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L8 ANSWER 1 OF 92 USPATFULL
ACCESSION NUMBER: 2000:167734 USPATFULL
TITLE: Up-converting reporters for biological and other **assays**
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APPLICATION INFO.:	US 1998-16402	19980130 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1997-962673, filed on 3 Nov 1997, now abandoned which is a continuation-in-part of Ser. No. US 1995-482203, filed on 7 Jun 1995, now patented, Pat. No. US 5698397 which is a division of Ser. No. US 1995-416023, filed on 30 Mar 1995, now patented, Pat. No. US 5674698 which is a continuation-in-part of Ser. No. US 1995-381006, filed on 30 Jan 1995, now abandoned which is a continuation of Ser. No. US 1992-946068, filed on 14 Sep 1992, now abandoned

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CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides methods, compositions, and apparatus for performing sensitive detection of analytes, such as biological macromolecules and other analytes, by labeling a probe molecule with an up-converting label. The up-converting label absorbs radiation from an illumination source and emits radiation at one or more higher frequencies, providing enhanced signal-to-noise ratio and the essential elimination of background sample autofluorescence. The methods, compositions, and apparatus are suitable for the sensitive detection of multiple analytes and for various clinical and environmental sampling techniques.

TI Up-converting reporters for biological and other **assays**

SUMM The invention relates generally to detectable labels and compositions

useful in **assay** methods for detecting soluble, suspended, or particulate substances or analytes such as proteins, carbohydrates, nucleic acids, bacteria, viruses, and eukaryotic. . .

SUMM . . . of such detection methods are widely used in biomedical research and clinical laboratory medicine. Examples of such detection methods inclide: **immunoassays**, immunochemical staining for microscopy, fluorescence-activated cell sorting (FACS), nucleic acid hybridization, water sampling, air sampling, and others.

SUMM For example, **radioimmunoassays** (RIA) have been among the most sensitive and specific analytical methods used for detecting and quantitating biological macromolecules. **Radioimmunoassay** techniques have been used to detect and measure minute quantities of specific analytes, such as polypeptides, drugs, steroid hormones, polynucleotides, metabolites, and tumor markers, in biological samples. **Radioimmunoassay** methods employ immunoglobulins labeled with one or more radioisotopes as the analytical reagent. Radiation (alpha, beta, or gamma) produced by. . .

SUMM Another alternative class of fluorophore that has been proposed are the down-converting luminescent **lanthanide** chelates (Soini and Lovgren (1987) CRC Crit. Rev. Anal. Chem. 18: 105; Leif et al. (1977) Clin. Chem. 23: 1492; Soini and Hemmila (1979) Clin. Chem. 25: 353; Seveus et al. (1992) Cytometry 13: 329). Down-converting **lanthanide** chelates are inorganic phosphors which possess a large downward Stokes shift (i.e., emission maxima is typically at least 100 nm greater than absorption maxima) which aids in the discrimination of **signal** from scattered excitation light. **Lanthanide** phosphors possess emission lifetimes that are sufficiently long (i.e., greater than 1 mu s) to permit their use in time-gated detection methods which can reduce, but not totally eliminate, noise caused by shorter-lived autofluorescence and scattered excitation light. Further, **lanthanide** phosphors possess narrow-band emission, which facilitates wavelength discrimination against background noise and scattered excitation light, particularly when a laser excitation source is utilized (Reichstein et al. (1988) Anal. Chem. 60: 1069). Recently, enzyme-amplified **lanthanide** luminescence using down-converting **lanthanide** chelates has been proposed as a fluorescent labeling technique (Evangelista et al. (1991) Anal. Biochem. 197: 213; Gudgin-Templeton et al.. . .

SUMM Until recently, down-converting **lanthanide** phosphors have had the significant disadvantage that their quantum efficiency in aqueous (oxygenated) solutions is so low as to render them unsuitable for cytochemical staining. Beverloo et al. (op.cit.) have described a particular down-converting **lanthanide** phosphor (yttrium oxysulfide activated with europium) that produces a signal in aqueous solutions which can be detected by time-resolved methods.. . .

SUMM However, the down-converting **lanthanide** phosphor of Beverloo et al. and the europium chelate of Seveus et al. require excitation wavelength maxima that are in. . .

SUMM The invention also provides biochemical **assay** methods for determining the presence and concentration of one or more analytes, typically in solution. The **assay** methods employ compositions of probes labeled with up-converting phosphors and/or up-converting dyes and apparatus for magnetically and/or optically trapping particles that comprise the analyte and the labeled probe. In one embodiment, a sandwich **assay** is performed, wherein an immobilized probe, immobilized on a particle, binds to a predetermined analyte, producing-an immobilization of the bound. . . of various sizes, colors, and/or shapes with distinct immobilized probe(s), and/or various excitation wavelengths, it is possible to perform multiple **assays** essentially simultaneously or contemporaneously. This multiplex advantage affords detection and quantitation of multiple

analyte species in a single sample. The **assay** methods are also useful for monitoring the progress of a reaction, such as a physical,

RWD FIG. 11 is a phosphor emission spectrum of sodium yttrium fluoride-**ytterbium/erbium** up-converting phosphor with an excitation laser source at a wavelength maximum of 977.2 nm; emission maximum is about 541.0 nm;

DRWD FIG. 12 is an excitation scan of the sodium yttrium fluoride-**ytterbium/erbium** phosphor excitation spectrum, with emission collection window set at 541.0 nm;

DRWD . . . 13 is a time-decay measurement of the phosphor luminescence at 541.0 nm after termination of excitation illumination for sodium yttrium fluoride-**ytterbium/erbium**;

DRWD FIG. 14 shows the phosphor emission intensity as a function of excitation illumination intensity for a sodium yttrium fluoride-**ytterbium/erbium** phosphor;

DRWD FIG. 20 shows schematically one embodiment of an sandwich **immunoassay** for detecting an analyte in a solution by binding the analyte (e.g., an antigen target) to a biotinylated antibody and. .

.

DRWD FIG. 23 show a schematic of a competitive homogeneous **assay** using phosphors as labels and fiber optic illumination at a capture surface.

DRWD FIG. 24 show a schematic of a competitive homogeneous antigen capture **assay** using phosphors as labels and a convergent illumination beam focused on the capture surface.

DRWD FIG. 25 shows a schematic of a homogeneous immunoprecipitation **assay** using phosphors as labels and a convergent illumination beam focused on the capture surface wherein the capture surface collects immunoprecipitates.

DETD . . . indicates the presence and/or location of probe. The invention encompasses organic and inorganic up-converting labels, but preferably employs up-converting inorganic **lanthanide** phosphors as **labels**. Thus, a typical label of the invention is a submicron-size up-converting **lanthanide** phosphor particle. The **label** can alternatively comprise a **lanthanide** ion in a chelate or cage compound.

DETD As used herein, the term "target" and "target analyte" refer to the object(s) that is/are **assayed** for by the methods of the invention. For example but not limitation, targets can comprise polypeptides (e.g., hGH, insulin, albumin),. . .

DETD . . . accomplished using various linkage chemistries, depending upon the nature of the specific probe. For example but not limitation, microcrystalline up-converting **lanthanide** phosphor particles may be coated with a polycarboxylic acid (e.g., Addition XW 330, Hoechst, Frankfurt, Germany) during milling and various. . .

DETD . . . mechanisms involved. Up-conversion has been found to occur in certain materials containing rare-earth ions in certain crystal materials. For example, **ytterbium** and **erbium** act as an activator couple in a phosphor host material such as bariumn-yttrium-fluoride. The **ytterbium** ions act as the absorber, and transfer energy non-ritdiatively to excite the **erbium** ions. The emission is thus characteristic of the **erbium** ion's energy levels.

DETD . . . aluminum garnet, gadolinium fluoride (GdF.sub.3), barium yttrium fluoride (BaYF.sub.5, BaY.sub.2 F.sub.8), and gadolinium oxysulfide. Suitable activator couples are selected from: **ytterbium/erbium**, **ytterbium**/thulium, and **ytterbium**/holmium. Other activator couples suitable for up-conversion may also be used. By combination of these host materials

. . . with the activator couples,. . . phosphors with at least three different emission spectra (red, green, and blue Nisible light) are provided. Generally, the absorber is **ytterbium** and the emitting center can be selected from: **erbium**, holmium, terbium, and thulium; however, other up-converting phosphors of the invention may contain other absorbers and/or emitters. The molar ratio. . .

DETD The optimum ratio of absorber (e.g., **ytterbium**) to the emitting center (e.g., **erbium**, thulium, or holmium) varies, depending upon the specific absorberemitter couple. For example, the absorber:emitter ratio for Yb:Er couples is typically. . .

DETD . . . invention typically have emission maxima that are in the visible range. For example, specific activator couples have characteristic emission spectra: **ytterbium-erbium** couples have emission maxima in the red or green portions of the visible spectrum, depending upon the phosphor host; **ytterbium**-holmium couples generally emit maximally in the green portion, **ytterbium** -thulium typically have an emission maximum in the blue range, and ytterbiumterbium usually emit maximally in the green range. For example,. . .

DETD For exemplification, but not to limit the invention, **ytterbium** (Yb)-**erbium**(Er)-doped yttrium oxysulfides luminesce in the green after excitation at 950 nm. These are non-linear phosphors, in that the **ytterbium** acts as an "antenna" (absorber) for two 950 nm photons and transfers its energy to **erbium** which acts as an emitter (activator). The critical grain size of the phosphor is given by the quantum yield for. . .

DETD TABLE I

Phosphor Material Compositions

Host Material	Absorber Ion	Emitter Ion	Color
Oxysulfides (O.sub.2 S)			
Y.sub.2 O.sub.2 S	**Ytterbium**	**Erbium**	Green
Gd.sub.2 O.sub.2 S	**Ytterbium**	**Erbium**	Red
La.sub.2 O.sub.2 S	**Ytterbium**	Holmium	Green
Oxyhalides (OX.sub.y)			
YOF	**Ytterbium**	Thulium	Blue
Y.sub.3 OCl.sub.7	Yterbium	Terbium	Green
Fluorides (F.sub.x)			
YF.sub.3	**Ytterbium**	**Erbium**	Red
GdF.sub.3	**Ytterbium**	**Erbium**	Green
LaF.sub.3	**Ytterbium**	Holmium	Green
NaYF.sub.3	**Ytterbium**	Thulium	Blue
BaYF.sub.5	**Ytterbium**	Thulium	Blue
BaY.sub.2 F.sub.8	**Ytterbium**	Terbium	Green
Gallates (Ga.sub.x O.sub.y)			
YGaO.sub.3	**Ytterbium**	**Erbium**	Red
Y.sub.3 Ga.sub.5 O.sub.12	**Ytterbium**	**Erbium**	Green
Silicates (Si.sub.x O.sub.y)			
YSi.sub.2 O.sub.5	**Ytterbium**	Holmium	Green
YSi.sub.3 O.sub.7	**Ytterbium**	Thulium	Blue

DETD . . . with zirconia and/or alumina balls for periods of up to about

48 hours or longer. Phosphor particles used in binding **assays** are typically about 3.0 to 0.01 .mu.m in diameter (or along the long axis if non-spherical), more usually about 2.0. . .

DETD . . . is not uniform, as milled particles result from random fracture of larger crystalline particles. Since the sensitivity of a detection **assay** using up-converting inorganic phosphors depends on the ability to distinguish between bound and unbound phosphor particles, it is preferable that. . .

DETD . . . is suitable for preparing much smaller phosphor particles (e.g., 0.1 .mu.m diameter or smaller), which may be advantageous for various **assay** formats.

DETD Binding **Assays**

DETD . . . dyes are used as reporters (i.e., detectable markers) to label binding reagents, either directly or indirectly, for use in binding **assays** to detect and quantitate the presence of analyte(s) in a sample. Binding reagents are labeled directly by attachment to up-converting. . . an up-converting inorganic phosphor).

Quantitative detection of the analyte-probe complex may be conducted in conjunction with proper calibration of the **assay** for each probe employed. A probe is conveniently detected under saturating excitation conditions using, for example, a laser source or. . .

DETD Specific binding **assays** are commonly divided into homogeneous and heterogeneous **assays**. In a homogeneous **assay**, the signal emitted by the bound labeled probe is different from the signal emitted by the unbound labeled probe, hence the two can be distinguished without the need for a physical separation step. In heterogeneous **assays**, the signal emitted from the bound and unbound labeled probes is identical, hence the two must be physically separated in order to distinguish between them. The classical heterogeneous specific binding **assay** is the **radioimmunoassay** (RIA) (Yalow et al. (1978) Science 200: 1245, which is incorporated herein by reference). Other heterogeneous binding **assays** include the radioreceptor **assay** (Cuatrecasas et al. (1974) Ann. Rev. Biochem. 43: 109), the sandwich **radioimmunoassay** (U.S. Pat. No. 4,376,110, which is incorporated herein by reference), and the antibodylectin sandwich **assay** (EP 0 166 623, which is incorporated herein by reference).

Heterogeneous **assays** are usually preferred, and are generally more sensitive and reliable than homogeneous **assays**.

DETD . . . it is often desirable to dilute the sample in one or more diluents that do not substantially interfere with subsequent **assay** procedures. Generally, suitable diluents are aqueous solutions containing a buffer system (e.g., 50 mM NaH.sub.2 PO.sub.4 or 5-100 mM Tris,. . .

DETD Binding conditions vary, depending upon the nature of the probe-label conjugate, target analite, and specific **assay** method. Thus, binding conditions will usually differ if the probe is a polynucleotide used in an in situ hybridization, in a Northern or Southern blot, or in solution hybridization **assay**. Binding conditions will also be different if the probe is an antibody used in an in situ histochemical staining method. . .

DETD . . . hormones, cytokines, or lymphokines, suitable binding conditions generally are those described in the art for performing the respective receptor-ligand binding **assay**.

DETD Various examples of suitable binding conditions useful in **immunoassays** and immunohistochemistry are discussed, for example, in Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor, N.Y. (1988), which. . .

DETD Binding **assays**, which include in situ hybridization, in situ binding **assays**, and immunohistochemical staining, are usually performed by first incubating the sample with a blocking or prehybridization solution, followed by incubating. . .

DETD . . . and coated with a probe (e.g., a specific antigen or antibody)

for use as a labeled probe in an immunodiagnostic **assay** or nucleic acid hybridization **assay** to detect an analyte in a sample, such as the presence of an antibody, virus, or antigen in a bloom. . .

DETD Sandwich Binding **Assays**

DETD Up-converting phosphors labels can be used as reporters for sandwich binding **assays** (U.S. Pat. No. 4,376,110, which is incorporated herein by reference). For example, a magnetic bead, such as a superparamagnetic immunobead. . .

DETD . . . example and not limitation, the following three brief examples are provided to explicate further possible applications of multiple analyte sandwich **assay** methods.

DETD . . . scan for presence and abundance of particular phosphors (by illuminating with excitation wavelength(s) and detecting emitted wavelengths). By performing binding **assays** under dilute conditions wherein an average of one analyte or less (e.g., lymphocyte) is bound per microbead, it is possible. . .

DETD . . . et al. (24-25 May 1991) Future Directions and Applications of Photodynamic Therapy, pp. 219; Folli et al. (Dec. 17, 1991) **Fluoresceine** Clinique 4; Braichotte et al. (May 1991) ENT-Clinic, Lausanne, Switzerland).

DETD . . . also be considered. One type of small up-converting inorganic phosphor consists of rare earth ions in chelates. The use of **lanthanide** chelates as reporters has been developed for biological **assays** as described above. This prior use of **lanthanide** chelates involved dowvn-conversion. That is, the emission light is at a wavelength which is longer than the excitation wavelength. Rare. . . diethylenetriaminepentaacetic acid (DTPA), tetraazacyclotetradecanetetraacetic acid (TETA), as well as antibiotics, natural chelating proteins, phthalocyanines, and cryptates. Methods for preparation of **lanthanide** chelates and their use in biological **assays** are described in the literature (Mukkala et al. (1989) Anal. Biochem. 176: 319, Hemrnila et al. (1984)Anal. Biochem. 137: 335,. . .

DETD Suitable ions for up-conversion in chelates include **erbium**, neodyrnium, thulium, holnium, and praseodymium. Other candidate ions include the other **lanthanide** elements, the actinide elements, and other metal elements. Stepwise excitation schemes suitable for up-conversion in **lanthanide** chelates are described in the literature on up-conversion lasers. Examples include up-conversion in **erbium** (Silversmith et al. (1986) J. Opt. Soc. Am. A3:128, and Macfarlane et al. (1989) Appl. Phys Lett. 54:2301), neodymium (Macfarlane. . .

DETD . . . are larger by two to three orders of magnitude. A few specific examples will be mentioned: in general cyanines, xanthenes, **rhodamines**, **acridines** and oxazines are well suited for this purpose. Blue dyes can also be used, but the excitation wavelength will be in the red. **Rhodamine** can be excited at 650 to 700 nm using two photons, and fluorescence is expected around 555 nm. Many IR. . .

DETD . . . at sufficiently high field intensities that can be routinely achieved using commercial laser sources. Specific examples are the excitation of **Rhodamine** around 650 to 700 nm, or BBQ excitation around 480 rm. Organic dyes absorbing in the red have to absorb. . .

DETD . . . ability to use higher illumination intensities without photobleaching or sample damage translates into larger potential signals, and hence more sensitive **assays**.

DETD The basic purpose of the instrumentation is to expose the up-converting phosphor particles of an **assay** sample to near-infrared (NIR) light and to measure the amount of visible light that is emitted.

DETD . . . involving a single photon energy. Further, it may be possible to achieve direct stepwise excitation of the emitting ion (the **erbium** ion in the example outlined above) without using energy

transfer from another absorbing ion (the **ytterbium** ion in the example) while taking advantage of resonant enhancement of intermediate levels. Additionally, the use of different wavelengths for. . .

DETD . . . stimulation beyond the sensitivity range of the detector array.

An example of such a phosphor would be Gadolinium oxysulfide: 10% **Erbium**. This phosphor is stimulated by 1.5-micron radiation and emits at 960 nm and 520 nm. The detector array is insensitive. . .

DETD . . . test chamber B1 is a near IR laser excitation source B2, a photomultiplier tube (PMT) detector B3, and a sample **assay** plate B4. In the preferred embodiment of this apparatus, **assay** plate B4 is a Terasaki HLA plate. This plate is preferred due to its small tapered sample wells which tend. . . configuration is still larger than the diameter of the laser beam. Furthermore, it is possible that the distribution of the **assay** material across the bottom of the well is not even. Because of these two factors, simply aiming the laser at. . .

DETD . . . laser B2 passes through a filter B5 and is focussed by a lens B6 onto an individual sample well of **assay** plate B4. Plate B4 is mounted on a pair of translators B7 which allow positioning in the horizontal and vertical. . .

DETD Since the emitted power scales as the square of the excitation intensity, diagnostics using upconverting phosphors perform better in a **microassay** format. Assuming a constant power output from the excitation source, the excitation power density increases proportionally with the decrease in. . .

DETD As described above, photophysical catalysis, diagnostic **assays** and other sampling techniques can take advantage of matching the absorption spectrum of a luminescent label with the emission spectrum. . . luminescent label, i.e., a label which emits radiation upon absorption of energy, represents a step beyond traditional photophysical catalysis and **assay** methods.

DETD . . . is excited by the energy emitted from the excitation label, and emits emission radiation which may be detected in an **assay** or used in photophysical catalysis or photodynamic therapy. Additionally, a matched label pair may have one or more transfer labels. . .

DETD The matched labels may be employed in any traditional **assay** or photophysical catalysis method. For example, in matched label **assays**, a sample suspected of containing a target analyte is contacted with matching up-converting and luminescent labeled probes. The up-converting labeled. . .

DETD The matched label **assays** may be carried out as homogeneous, heterogeneous or competitive **assays**. However, when using matching labeled probes in a homogeneous **assay**, it is preferred that the matching labels are attached to two probes to form distinct labeled-probe pairs which bind to. . .

DETD Matched label photophysical catalysis can be conducted in a manner similar to the matched label **assays** described above except that the energy from the emission label may be used to produce localized intense electromagnetic radiation for. . .

DETD Both the matched label photophysical catalysis and **assays** of the invention may employ a multitude of different matched labels and/or different probes within a single sample. By varying. . .

DETD The following four embodiments outline the ability to conduct multiple **assays** within a single sample:

DETD . . . are in close proximity, e.g., are bound to the same analyte. Thus, different target analytes may be detected in an **assay**. For example, different matched label pairs may be attached to two distinct probes which bind to two different target analytes. Then, for an **assay** method, by exciting the excitation label and

observing for emission radiations from the emission labels, the presence
of one or. . .

DETD . . . each having distinct excitation labels and distinct emission labels may be used in a single sample. For example, in an **assay** method, by exciting the first excitation label and observing emission radiation from its matched label, a first analyte may be. . .

DETD . . . probe having one excitation label may be matched with at least two probes having distinct emission labels. Thus, in an **assay**, different target analytes may be detected within a single sample by exciting the excitation label and then observing the different. . .

DETD . . . more emission labels which emit a detectable emission radiation
and at least two distinct excitation labels. For example, in an **assay** on a single sample, different target analytes may be detected by separately exciting the distinct excitation labels and observing the. . .

DETD Matched labels may also be used in other **assay** formats, (for example a competitive **assay**), where a first labeled probe of a matched labeled probe pair binds with a target analyte to form an initial. . .

DETD Any luminescent labels used in conventional **assays** or, as described above, photophysical catalysis methods may be up-converting or
down-converting, phosphorescent or fluorescent materials. The luminescent labels which. . .

DETD . . . compositions may be in the form of a kit comprising all of the essential ingredients required to conduct the desired **assay** or photophysical catalysis method. For example, the kit may contain matching up-converting labeled probes and luminescent labeled probes. The **assay** kit is presented in a convenient, commercially packaged form. The kit can be presented as a composition or as an admixture depending upon the compatibility of the labeled probes. For example an **assay** kit can be a packaged combination having one or more containers, devices or the like holding the labeled probes and other materials necessary for particular **assay**, and usually including written instructions for the performing the **assay**.

DETD Up-converting phosphor particles comprising sodium yttrium fluoride doped with **ytterbium-erbium** were milled to submicron size, fractionated by particle size, and coated with polycarboxylic acid. Na(Y.sub.0.80 Yb.sub.0.18 Er.sub.0.02)F.sub.4 was chosen for. . .

DETD . . . detection sensitivity (Table III). It should be noted that 10.sup.-15 to 10.sup.-18 M is the normal range of enzyme-linked surface **assays**.

DETD A series of IgG/anti-IgG samples for demonstrating the capabilities of the up-converting phosphor reporters in a immunosorbant **assay** format was prepared. These samples consisted of six individual wells (positive samples) coated with antigen (mouse IgG) and bovine serum. . .

DETD . . . resuspended in a 3 .mu.g/.mu.L BSA solution in PBS for post-coating. The resulting BSA/PSA resuspension was used directly for the **assay**.

DETD . . . to delineate further the parameters for up-converting phosphors
as biochemical reporters, biological linkers were attached to phosphor particles. Sodium yttrium fluoride-**ytterbium/erbium** phosphor particles were coated with streptavidin. The excitation and emission spectral properties of the phosphor alone and the phosphor coated. . . phosphors were then specifically bound to biotinylated magnetic beads, demonstrating the applicability of linker-conjugated inorganic phosphors as reporters in biochemical **assays**, such as **immunoassays**, immunohistochemistry, nucleic acid hybridizations, and other **assays**. Magnetic bead technology allows for the easy separation of biotin-bound streptavidin-coated phosphor from a solution, and is particularly well-suited for sandwich

assays wherein the magnetic bead is the solid substrate.

DETD Advantageously, streptavidin-biotin chemistry is widely used in a variety of biological **assays**, for which up-converting phosphor reporters are suited. FIG. 20 shows schematically, for example and not limitation, one embodiment of an **immunoassay** for detecting an analyte in a solution by binding the analyte (e.g., an antigen target) to a biotinylated antibody, wherein. . .

DETD Background signals were determined in two biological samples for determination of potential background in **immunoassays**. Sputum and urine were used as samples in the same apparatus as used for the phosphorescence sensitivity measurements (supra). No. . .

DETD An upconverting ytrium-**ytterbium-erbium** (Y.sub.0.86 Yb.sub.0.08 Er.sub.0.06) oxysulfide (O.sub.2 S) phosphor was linked to avidin by the following procedure:

DETD . . . process was repeated. The washed pellet was resuspended in 1.0 mL of phosphate buffered saline and used directly in diagnostic **assays** as described below.

DETD . . . the paramagnetic beads in the absence of DNA. Samples 5 and 6 show results of FITC-labeled avidin used to validate **assay**.

DETD TABLE V

Upconverting Phosphor Nucleic Acid Diagnostic **Assay** Results

Sample	DNA	Reporter	PMT Signal (V @ 546 nm)	PMT Signal (V @ 514 nm)
1	DNA labeled with	Avidin	6.1297. . .	

DETD HOMOGENEOUS **ASSAYS**

DETD The multiphoton activation process characteristic of upconverting phosphors can be exploited to produce **assays** that require no sample washing steps. Such diagnostic **assays** that do not require the removal of unbound phosphor labels from the sample are herein termed homogeneous **assays**, and can also be termed pseudohomogeneous **assays**.

DETD Homogeneous **Assay** Example 1

DETD One embodiment of a homogeneous **assay** consists of the use of an upconverting phosphor label linked to an appropriate probe (e.g., an antibody or DNA). The. . . reactive with target, polynucleotide that binds target) that is itself linked to the capturing surface (such as in a sandwich **immunoassay**, for example).

DETD Examples of suitable homogeneous **assay** formats include, hut are not limited to, immunodiagnostic sandwich **assays** and antigen and/or antibody surface competition **assays**.

DETD Homogeneous **Assay** Example 2

DETD Up-conversion has been performed in rare earth chelate and rare earth salt solutions. Chelate of **erbium** and neodymium have been prepared with ethylenediaminetetraacetic acid (EDNA) and dipicolinic acid (DPA). The **erbium** chelate were pumped using light near 793.5 nm from a Ti:sapphire laser (the excitation scheme of Macfarlane (1989) Appl. Phys.. . .

CLM What is claimed is:

4. The composition according to claim 1, wherein the composition is in the form of an **assay** kit.

L8 ANSWER 2 OF 92 USPATFULL

ACCESSION NUMBER: 2000:164279 USPATFULL

TITLE: Site-specific bioconjugates of chemokines and their use in **assays**

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LEGAL REPRESENTATIVE: Heslin & Rothenberg, P.C.

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NUMBER OF DRAWINGS: 8 Drawing Figure(s); 4 Drawing Page(s)

LINE COUNT: 713

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention encompasses polypeptides that comprise a chemokine receptor binding sequence and are useful in determining the affinity of a compound for a chemokine receptor. Substitution of one of the amino acids of the C-terminal region of the polypeptide with a cysteine enables the polypeptide to be detectably labelled without loss of receptor binding activity and without the problems inherent in radioiodine labelling. Methods for use of the polypeptides in competitive binding **assays** are also disclosed.

TI Site-specific bioconjugates of chemokines and their use in **assays**

AB . . . receptor binding activity and without the problems inherent in radioiodine labelling. Methods for use of the polypeptides in competitive binding **assays** are also disclosed.

SUMM . . . antagonists are candidates as therapeutic agents for IL-8-dependent disorders. For example, IL-8 labelled with .sup.125 I has been used in **assays** for binding to CHO membranes to determine IL-8 antagonists, as described in WO 9625157. However, .sup.125 I is a dangerous. . .

SUMM **Lanthanides** are fluorescent **labels** that have been used primarily in clinical diagnostic kits in standard **immunoassay** formats; i.e., they have been used to label antibodies for use in such kits. For example, they are conventionally used in time-resolved fluorescent **assays** for clinical screening. Multiple **lanthanide labels** (Europium, Samarium, Terbium, and Dysprosmium) can be used in the same **assay**, and distinguished by their differing emission wavelength/decay time fluorescent profiles. Thus, different analytes can be determined in the same **assay** using these different labels.

SUMM The fluorescent properties of **lanthanide** probes make them particularly useful in biological **assays** (Soini, E. and Loevgren, T. (1987) CRC Crit. Rev. Anal. Chem. 18, 105-153). Specifically, the extended fluorescence lifetime of **lanthanides** allows measurement of **signal** to be made after the decay of shorter-lived background fluorescence originating from either the biological sample or the plastic support. For example, Eu-labelled anti-phosphotyrosine has been used in a sensitive protein tyrosine kinase **assay** (Braunwalder et al., (1996) Anal. Biochem. 238, 159-164). Also, Takeuchi, T., et al., ((1995) Anal. Chem. 67, 2655-8) have described a **lanthanide-labelled** benzodiazepine used in receptor binding **assays**.

SUMM **Lanthanide** reagents would appear to be potentially useful in high throughput screening of such libraries to measure the binding of chemokines to their receptors. Conjugation of **lanthanide** chelates, and other detectable **labels**, to chemokines, however, can, and has, resulted in substantial loss of binding affinity.

SUMM . . . amino acid position no. 1 under mild periodate oxidation conditions and reacting the resultant glyoxylyl with an amino-oxy derivative of **fluorescein**. The resultant **fluorescein** -labelled IL-8 had a 10-fold lower affinity than [.sup.125 I]IL-8 for IL-8 receptors. Thus, binding of IL-8 was substantially lost with **fluorescein** isothiocyanate labeling.

SUMM IL-8 can be prepared containing **lanthanide** fluorescent **labels** conjugated to IL-8 at available lysine groups. This method, however, can modify lysine residues that are critical to the receptor-ligand interaction. Thus, while the resulting reagent may be useful for a competitive **immunoassay** for determining the amount of IL-8 in a sample, it is less acceptable for use in a competitive **assay** to measure receptor/ligand binding.

SUMM It is also an object of the invention to provide an **assay** that is easily adaptable for high throughput screening of potential CC and CXC chemokine receptor ligands.

DRWD FIG. 1B shows cysteine conjugated to **fluorescein**.

DRWD FIG. 2 depicts the sulfhydryl chemistry of **lanthanide-labelled** IL-8. In FIG. 2A nucleophilic attack of thiol or thiolate upon the alpha carbon of a monosubstituted haloacetamide under neutral. . .

DETD . . . and any suitable fluorescent label can be used. Examples include cyanine dyes such as Cy-5, Cy-5.5, and Cy7 (Amersham Corp.), **fluorescein**, **rhodamine** and Texas red. FIGS. 1B and 1C show embodiments of the invention wherein cysteine is conjugated to **fluorescein** or Cy5.

DETD Where a cell based **assay** is to be performed using a fluorescent label, it is preferred that the label fluoresce at a relatively high wavelength,. . .

DETD The preferred fluorescent **labels** are **lanthanides**. Such **labels**, and reagents for **labelling** IL-8 and other chemokines, are commercially available, for example from EG&G Wallac. The labelling reagent used for conjugating the cysteine. . .

DETD . . . use of the labelled polypeptides of the invention in methods for determining ligands that bind to the chemokine receptor. Such **assays** are well known in the art using, e.g., iodinated IL-8. An example of such an **assay** is shown below. For example, in a method of the invention, a compound to be screened for affinity for IL-8. . .

DETD The **assay** can be conducted in a homogeneous manner (i.e., without a liquid separation step) or in a heterogeneous manner (i.e., including a separation step). A preferred homogeneous **assay** is described in parent application Ser. No. 08/828,280, filed on Jun. 3, 1997, the contents of which have been incorporated by reference herein. Another homogeneous **assay** is described in Ser. No. 08/553,056, published as WO 97/16569.

DETD . . . compounds to be screened, preferably at least about 96 compounds, such as when using a 96 well microtitre plate. Such

assays can also be performed in the 1536 well plate described in U.S. patent application Ser. No. 60/037,636, filed Feb. 18,. . .

DETD It is preferred that the compounds **assayed** in the high throughput method be derived from combinatorial libraries on polymer beads. By synthesizing sufficient compound on each bead for a few **assays**, compound handling is reduced or eliminated.

DETD Preferably, the library compounds are eluted from the beads and evaporated to dryness in microtiter plates in preparation for the **assay**. Compounds on beads can be released by photocleavage, or another type of cleavage. Cleavage of photocleavable linkers is preferred. Such. . .

DETD Formation of **Lanthanide Labelled** IL-8 and Use in **Assay**

DETD . . . from Dupont/NEN, Research Products (Boston, Mass.). CHO K1 cells were obtained from the American Type Culture Collection (ATCC). Bradford protein **assay** kit was from Pierce Chemical Co. (Rockford, Ill.). Binding buffer used in the ligand binding **assay** contained 25 mM HEPES, 11.5 mM KCl, 11.5 mM NaCl, 6 mM MgSO.sub.4, 1.8 mM CaCl.sub.2 and 0.25% BSA.

DETD Ligand Binding **Assay**

DETD CHO cells expressing either CXC R1 or CXC R2 were seeded in a 96-well tissue culture plate (Costar) for Europium-based **assays** or in a 96-well opaque-wall/black mask tissue culture plate (EG & G Wallac) for .sup.125 I-based **assays**. Confluent cells (.about.40,000 cells/well) were washed with binding buffer and incubated with ligands in a 50 ml/well reaction volume for. . .

DETD . . . to have identical binding properties to wild type IL-8 that was prepared by the same expression and purification scheme when **assayed** for its ability to compete with [.sup.125 I]IL-8 on recombinantly expressed CXCR1 or CXCR2. Results are shown in FIG. 4.

DETD . . . IL-8 on a 16% SDS-PAGE analysis and displayed a K.sub.i of 2 nM in a conventional [.sup.125 I]IL-8 ligand displacement **assay** employing CXC R2 receptor. This was similar to the value obtained using [.sup.125 I]IL-8 ligand. Thus, Cy5 labelled IL-8 according. . .

CLM What is claimed is:

14. The polypeptide of claim 13 wherein said **label** is a **lanthanide** chelate.

17. The polypeptide of claim 16 wherein said **label** is a **lanthanide** chelate.

19. The method of claim 18 wherein said **assay** comprises a homogeneous **assay**.

20. The method of claim 18 wherein said **assay** comprises a heterogeneous **assay**.

21. In a competitive **assay** for determining binding of a compound to a receptor for which interleukin-8 (IL-8) is a ligand, wherein said compound and. . .

L8 ANSWER 11 OF 92 USPATFULL

ACCESSION NUMBER:	2000:80847 USPATFULL
TITLE:	Labeling reactants and their use
INVENTOR(S):	Takalo, Harri, Turku, Finland Hovinen, Jari, Turku, Finland Mukkala, Veli-Matti, Kaarina, Finland Liitti, Paivi, Turku, Finland Mikola, Heikki, Turku, Finland
PATENT ASSIGNEE(S):	Wallac Oy, Turku, Finland (non-U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 6080839	20000627
APPLICATION INFO.:	US 1998-104219	19980625 (9)

DOCUMENT TYPE:	Utility
PRIMARY EXAMINER:	Ceperley, Mary E.
LEGAL REPRESENTATIVE:	Lydon & Brown, LLP
NUMBER OF CLAIMS:	22
EXEMPLARY CLAIM:	7
NUMBER OF DRAWINGS:	2 Drawing Figure(s); 2 Drawing Page(s)
LINE COUNT:	673

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to a novel labeling reactant, suitable for labeling of a biospecific binding reactant using solid-phase synthesis. The invention further concerns new labeling methods. The novel labeling reactant has the formula (I) ##STR1## wherein -A- is a bivalent aromatic structure capable of absorbing light or energy and transferring the excitation energy to a **lanthanide** ion after the product made by the said solid-phase synthesis has been released from the used solid support, deprotected and converted to a **lanthanide** chelate;

-G- is a bridge replacing a hydrogen atom in A;

R is a protected amino acid residue --CH(NHX)COOH, where X is a transient protecting group, or its active ester, where said ester is e.g. an N-hydroxysuccinimido, p-nitrophenol or pentafluorophenol ester; and

R' is --COOR'" where R'" is an alkyl of 1 to 4 carbon atoms, phenyl or benzyl, which phenyl or benzyl can be substituted or unsubstituted.

AB . . . wherein -A- is a bivalent aromatic structure capable of absorbing light or energy and transferring the excitation energy to a **lanthanide** ion after the product made by the said solid-phase synthesis has been released from the used solid support, deprotected and converted to a **lanthanide** chelate;

SUMM . . . agents for proteins. Radioactive .sup.153 Gd, .sup.111 In etc. can be used for in vivo tumor localization and radioimmunotherapy. Using **lanthanide** ions and a separate luminescence enhancement step these published monomers can be used as luminescent labels but a separate enhancement step is needed to produce the luminescence to be detected. During the luminescence enhancement the **lanthanide** has to be dissociated from the label and then spatial information will be lost.

SUMM . . . G., Hughes, S. G., Mecklenberg, S. L., Meyer, T. J. and Erickson, B. W., 1995, Tetrahedron, 51, 1093, WO 96/03409). **Fluorescein** and other organic chromophores were used in those

studies. However, such labels and labeled biomolecules suffer from many commonly known. . .

SUMM In the specific binding **assays**, such as e.g. **immunoassays**, DNA hybridization **assays**, receptor-binding **assays**, and cellular binding **assays**, generally very low concentrations of the analytes to be measured are present. Therefore, various labeling compounds have been developed that allow the labeled reactant to be detected and quantitated with high sensitivity. In **immunoassays** and DNA hybridization **assays**, time-resolved luminescence spectroscopy using **lanthanide** chelates is well known. (e.g. I. Hemmila, T. Stahlberg, and P. Mottram (eds.), "Bioanalytical Applications of Labeling Technologies", Wallac, Turku, 1994). Stable photoluminescent (in this context simply referred to as luminescent) **lanthanide** chelates also have other applications, e.g. fluorescence microscopy and cytometry. Therefore, a number of attempts have been made to develop.

.

SUMM . . . easier labeling methods. By preparing suitable ligand structures together with solid-phase synthesis the labeling of small molecules with directly luminescent **lanthanide** chelates is achievable.

SUMM -A- is a bivalent aromatic structure capable of absorbing light or energy and transferring the excitation energy to a **lanthanide** ion after the product made by the said solid-phase synthesis has been released from the used solid support, deprotected and converted to a **lanthanide** chelate;

SUMM . . . wherein -A- is a bivalent aromatic structure capable of absorbing light or energy and transferring the excitation energy to a **lanthanide** ton after the product made by the said solid-phase synthesis has been released from the used solid support, deprotected and converted to a **lanthanide** chelate;

SUMM c) converting the product from step b) to a **lanthanide** chelate, and

SUMM . . . in step a) is not part of the final biospecific binding specific reactant, attaching said biospecific binding reactant to the **lanthanide** chelate obtained in step c).

SUMM . . . wherein -A- is a bivalent aromatic structure capable of absorbing light or energy and transferring the excitation energy to a **lanthanide** ion after the product made by the said solid-phase synthesis has been released from the used solid support, deprotected and converted to a **lanthanide** chelate;

SUMM f) converting the product from step e) to a **lanthanide** chelate.

DETD . . . not limited to antigens, haptens, peptides, receptor ligands, drugs or PNA oligomers which are used in specific bioaffinity based binding **assays**, such as **immunoassays**, DNA hybridization **assays**, receptor binding **assays**, immunocytochemical or immunohistochemical **assays** utilizing fluorometric or time-resolved fluorometric determination of the specific luminescence.

DETD According to a preferred embodiment of the invention the **lanthanide** chelate is a europium(III), terbium(III), samarium(III) or dysprosium(III) chelate.

DETD . . . form a luminescent labeled biospecific binding reactant after releasing the product from solid support, deprotection and addition of a suitable **lanthanide** ion. The **labeling** technique of the present invention has many advantages over conventional labeling in liquid phase, such as high yields of easily. . . improved robustness, upscaling and repeatability of synthesis and labeling of biospecific binding reactant with an exact number of stable luminescent

lanthanide chelates is possible. Although many of the advantages relate to solid-phase labeling, the compounds of the present invention can also. . .

DETD aromatic structure capable of absorbing light or energy and transferring the excitation energy to a **lanthanide** ion after the product made by the said solid-phase synthesis has been released from the used solid support, deprotected and converted to a **lanthanide** chelate,

DETD . . . on the solid phase and then cleaved therefrom whereafter the cleaved product, before or after having been converted into a **lanthanide** chelate, is finally reacted in solution with the biospecific binding reactant.

DETD . . . solid support and deprotected. Purification can be performed by HPLC techniques. Finally the purified ligand is converted to the corresponding **lanthanide**(III) chelate by addition of known amount of **lanthanide**(III) ion, (Mukkala, V.-M. et al. Helv. Chim. Acta 1993, 76, 1361 and M. Kwiatkowski, M. Samiotaki, U. Lamminmaki, V.-M. Mukkala. . .

CLM What is claimed is:

. . . wherein -A- is a bivalent aromatic structure capable of absorbing light or energy and transferring the excitation energy to a **lanthanide** ion after the product made by the said solid-phase synthesis has been released from the used solid support, deprotected and converted to a **lanthanide** chelate; -G- is a bridge replacing a hydrogen atom in A and is formed of one to ten moieties, each. . .

. . . wherein -A- is a bivalent aromatic structure capable of absorbing light or energy and transferring the excitation energy to a **lanthanide** ion after the product made by the said solid-phase synthesis has been released from the used solid support, deprotected and converted to a **lanthanide** chelate; -G- is a bridge replacing a hydrogen atom in A and is formed of one to ten moieties, each. . . solid phase, converting R' to --COOH, and removing optional protection groups, c) converting the product from step b) to a **lanthanide** chelate, and d) in case the functional group mentioned in step a) is not part of the final biospecific binding specific reactant, attaching said biospecific binding reactant to the **lanthanide** chelate obtained in step c).

. . . wherein -A- is a bivalent aromatic structure capable of absorbing light or energy and transferring the excitation energy to a **lanthanide** ion after the product made by the said solid-phase synthesis has been released from the used solid support, deprotected and converted to a **lanthanide** chelate; -G- is a bridge replacing a hydrogen atom in A and is formed of one to ten moieties, each. . . product to the solid phase, and removing optional protecting groups, and

f) converting the product from step e) to a **lanthanide** chelate.

. . . 14. The method according to claim 13 wherein step f) is followed by reacting the biospecific binding reactant with the **lanthanide** chelate obtained in step f), to obtain the labeled biospecific binding reactant.

L8 ANSWER 23 OF 92 USPATFULL
ACCESSION NUMBER: 1999:159762 USPATFULL
TITLE: Homogeneous luminescence **assay** method based on energy transfer
INVENTOR(S): Latva, Martti, Turku, Finland
Hemmila , Ilkka, Kaarina, Finland
Blomberg, Kaj, Turku, Finland
Hurskainen, Pertti, Piispanristi, Finland
PATENT ASSIGNEE(S): Wallac Oy, Turku, Finland (non-U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5998146	19991207
APPLICATION INFO.:	US 1998-116763	19980717 (9)

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Marschel, Ardin H.
LEGAL REPRESENTATIVE: Lydon & Brown, LLP
NUMBER OF CLAIMS: 13
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 4 Drawing Figure(s); 4 Drawing Page(s)
LINE COUNT: 952
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention is a luminescence energy transfer based bioaffinity **assay** comprising a first group labeled with an energy donating compound (donor) and a second group labeled with an energy accepting compound (acceptor), wherein the donor is a luminescent **lanthanide** chelate having a long excited state lifetime and the acceptor is either a luminescent compound having a short excited state lifetime or a nonluminescent compound. The increase or decrease, respectively, in the energy transfer from the donor to the acceptor resulting from shortening or lengthening, respectively, of the distance between the said groups, is measured. Characteristic for the invention is that the **lanthanide** energy emission and the acceptor energy absorption do not essentially or not at all overlap each other.

L8 ANSWER 24 OF 92 USPATFULL
ACCESSION NUMBER: 1999:159751 USPATFULL
TITLE: Energy transfer hybridization **assay** using intercalators and **lanthanide** metals
INVENTOR(S): Rabbani, Elazar, New York, NY, United States
Hurley, Ian, Staten Island, NY, United States
PATENT ASSIGNEE(S): Enzo Diagnostics, Inc., Farmingdale, NY, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5998135	19991207
APPLICATION INFO.:	US 1995-486053	19950607 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1994-194215, filed on 9 Feb 1994, now abandoned which is a continuation of Ser. No. US 1989-314995, filed on 24 Feb 1989, now abandoned
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Horlick, Kenneth R.
LEGAL REPRESENTATIVE: Fedus, Esq., Ronald C.
NUMBER OF CLAIMS: 53
EXEMPLARY CLAIM: 26
LINE COUNT: 876

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Disclosed is a nucleic acid hybridization **assay** composition for detecting the presence of absence of a target oligo- or polynucleotide in a sample. The composition comprises: a solid matrix having at least one surface which is substituted with a first intercalator capable of binding dsDNA dsRNA, or DAN-RNA hybrids; a second intercalator, which may or may not comprise at least one fluorophore, said intercalator or said fluorophore each acting as either an energy donor or an energy acceptor; and an oligo- or polynucleotide probe which is specifically hybridizable with the target oligo- or polynucleotide and has directly or indirectly bound thereto, at least one **lanthanide** metal chelate or at least one fluorophore, each acting as either an energy donor or an energy acceptor. Also disclosed are a method and kit for its use.

L8 ANSWER 25 OF 92 USPATFULL

ACCESSION NUMBER: 1999:145914 USPATFULL

TITLE: Systems and methods for rapidly identifying useful chemicals in liquid samples

INVENTOR(S): Stylli, Chari, San Diego, CA, United States
Beckey, Samuel S., San Diego, CA, United States
Shumate, Christopher Bentley, La Jolla, CA, United States
Coassin, Peter J., Encinitas, CA, United States

PATENT ASSIGNEE(S): Aurora Biosciences Corporation, San Diego, CA, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5985214	19991116
APPLICATION INFO.:	US 1997-858016	19970516 (8)

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Achutamurthy, Ponnathapura
ASSISTANT EXAMINER: Ricigliano, Joseph W.
LEGAL REPRESENTATIVE: Fish & Richardson P.C.
NUMBER OF CLAIMS: 30
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 24 Drawing Figure(s); 29 Drawing Page(s)
LINE COUNT: 3617

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides for systems and methods that utilize automated and integratable workstations for identifying chemicals having useful activity. The present invention is also directed to chemical entities and information (e.g., chemical or biological activities of chemicals) generated or discovered by operation of workstations of the present invention. The present invention includes automated workstations that are programmably controlled to minimize processing times at each workstation and that can be integrated to minimize the processing time of the liquid samples from the start to finish of the process.

L8 ANSWER 37 OF 92 USPATFULL

ACCESSION NUMBER: 1999:43404 USPATFULL

TITLE: Up-converting reporters for biological and other **assays** using laser excitation techniques

INVENTOR(S): Zarling, David A., Menlo Park, CA, United States
Rossi, Michel J., Lausanne, Switzerland
Peppers, Norman A., Belmont, CA, United States
Kane, James, Lawrenceville, NJ, United States
Faris, Gregory W., Menlo Park, CA, United States
Dyer, Mark J., San Jose, CA, United States
Ng, Steve Y., San Francisco, CA, United States
Schneider, Luke V., Half Moon Bay, CA, United States

PATENT ASSIGNEE(S): SRI International, Menlo, CA, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5891656	19990406
APPLICATION INFO.:	US 1997-887428	19970702 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1995-416023, filed on 30 Mar 1995, now patented, Pat. No. US 5674698 which is a continuation-in-part of Ser. No. US 1995-381006, filed on 30 Jan 1995, now abandoned which is a continuation of Ser. No. US 1992-946068, filed on 14 Sep 1992, now abandoned

DOCUMENT TYPE: Utility

PRIMARY EXAMINER: Housel, James C.

ASSISTANT EXAMINER: Portner, Ginny Allen

LEGAL REPRESENTATIVE: Morgan, Lewis & Bockius LLP

NUMBER OF CLAIMS: 6

EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 41 Drawing Figure(s); 32 Drawing Page(s)

LINE COUNT: 3491

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides methods, compositions, and apparatus for performing sensitive detection of analytes, such as biological macromolecules and other analytes, by labeling a probe molecule with an up-converting label. The up-converting label absorbs radiation from an illumination source and emits radiation at one or more higher frequencies, providing enhanced signal-to-noise ratio and the essential elimination of background sample autofluorescence. The methods, compositions, and apparatus are suitable for the sensitive detection of multiple analytes and for various clinical and environmental sampling techniques.

L8 ANSWER 42 OF 92 USPATFULL
ACCESSION NUMBER: 1998:159712 USPATFULL
TITLE: Analyte **assay** using a trifunctional conjugate
INVENTOR(S): Oh, Chan S., Diamond Bar, CA, United States
Sternberg, James C., Fullerton, CA, United States
PATENT ASSIGNEE(S): Beckman Instruments, Inc., Fullerton, CA, United States
(U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5851778	19981222
APPLICATION INFO.:	US 1997-832143	19970402 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1995-410014, filed on 22 Mar 1995, now patented, Pat. No. US 5661019 which is a continuation of Ser. No. US 1992-911827, filed on 10 Jul 1992, now abandoned which is a division of Ser. No.
US 1991-768118, filed on 30 Sep 1991, now patented, Pat. No. US 5168057 which is a continuation of Ser. No.
US 1987-103093, filed on 30 Sep 1987, now abandoned
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Naff, David M.
LEGAL REPRESENTATIVE: May, William H.; Kivinski, Margaret A.
NUMBER OF CLAIMS: 46
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 12 Drawing Figure(s); 12 Drawing Page(s)
LINE COUNT: 2608
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A trifunctional conjugate is provided having three chemical moieties attached through a spacer moeity. At least two of the chemical moieties are relatively small molecules, usually less than about 7,000 Daltons in size. The spacer moiety is selected to impart certain steric properties to the conjugate. In one embodiment, the binding of a macromolecular specific binding partner to one of the chemical moieties sterically inhibits the binding of a different macromolecule to another chemical moiety. In another embodiment, the binding of a first chemical moiety to a macromolecule restricts the subsequent binding of a second chemical moiety to a proximate location on the same macromolecule. The three chemical moieties are preferably a nitrophenylazido residue, a phenyl boronic acid residue, and a solid support or a label such as biotin. The spacer is preferably cysteine, lysine, glutamic acid, pyroglutamic acid, S-acetylmercaptosuccinic anhydride or .omega.-carbobenzoxylysine. The conjugate is useful in **immunoassays** and for targeted labeling of proteins. In an **assay**, a sample is contacted with the conjugate, a limited quantity of analyte binding partner and an excess of small molecule binding partner. Presence of the analyte is determined by detecting the amount of analyte binding partner diverted away from analyte attached to the spacer of the conjugate.

L8 ANSWER 46 OF 92 USPATFULL
ACCESSION NUMBER: 1998:134918 USPATFULL
TITLE: Homogeneous **fluorassay** methods employing

fluorescent background rejection and water-soluble rare
earth metal chelates

INVENTOR(S): Wieder, Irwin, 459 Panchita Way, Los Altos, CA, United States 94022
Hale, Ron L., 17085 Skyline Blvd., Woodside, CA, United
States 94062

	NUMBER	DATE
PATENT INFORMATION:	US 5830769	19981103
APPLICATION INFO.:	US 1996-732871	19961015 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1994-338285, filed on 10 Nov 1994, now abandoned which is a continuation of Ser.
No. US 1993-35516, filed on 22 Mar 1993, now abandoned which is a continuation of Ser. No. US 1986-875287, filed on 17 Jun 1986, now abandoned which is a continuation-in-part of Ser. No. US 1985-712779, filed on 18 Mar 1985, now patented, Pat. No. US 4761481
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Green, Lora M.
LEGAL REPRESENTATIVE: Adduci, Mastriani & Schaumberg, L.L.P.
NUMBER OF CLAIMS: 22
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Figure(s); 1 Drawing Page(s)
LINE COUNT: 1406
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Homogeneous **assays** for determining quantitatively the extent of a specific binding reaction can be carried out effectively on very dilute solutions using measurements of fluorescence if a fluorescence measurement scheme that is capable of rejecting short-lived background fluorescence is employed and if the fluorescent group being measured has the following properties: a. the group being measured must be a rare earth metal chelate complex combination; b. the chelate must be water-soluble; c. the complex combination must also be stable in extremely dilute aqueous solutions, that is, the measured chelate must have at least one ligand having a metal-to-ligand binding constant of at least about 10.sup.13 M.sup.-1 or greater and it must have a fluorescent emission that is long-lived compared to the longest decay lifetime of ambient substances and have a half life of from 0.01 to 50 msec.

L8 ANSWER 56 OF 92 USPATFULL
ACCESSION NUMBER: 1998:1627 USPATFULL
TITLE: Reduction of background in noncompetitive binding **assays**
INVENTOR(S): Piran, Uri, Sharon, MA, United States
Livshin, Laurie Ann, Sharon, MA, United States
Martinelli, Richard A., Brighton, MA, United States
Riordan, William J., Mansfield, MA, United States
Unger, John T., Medfield, MA, United States
PATENT ASSIGNEE(S): Chiron Diagnostics Corporation, Walpole, MA, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5705338	19980106
APPLICATION INFO.:	US 1995-434743	19950504 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1993-121806, filed on 15 Sep 1993, now patented, Pat. No. US 5445936
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Knode, Marian C.
ASSISTANT EXAMINER: Wortman, Donna C.
LEGAL REPRESENTATIVE: Morgenstern, Arthur S.; Blackburn, Robert P.
NUMBER OF CLAIMS: 7
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 9 Drawing Figure(s); 8 Drawing Page(s)
LINE COUNT: 883
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Novel non-competitive **assay** techniques have been developed which not only improve sensitivity, but also are convenient and less susceptible to interfering factors. They are compatible with existing instruments and can be run in one or more test tubes. The analyte is reacted with labeled specific binder, after which the mixture is reacted with (1) an insoluble material attached to an analyte derivative and (2) a solid phase carrying a binder. The solid phase is then separated, and the label attached to the solid phase is measured. Variations of the procedure include the use of a reversible bridge for attaching the insoluble material to the analyte mimic and the conduct of the **assay** in various porous media, such as paper, chromatographic and electrophoretic media, and dipsticks.

L8 ANSWER 60 OF 92 USPATFULL
ACCESSION NUMBER: 97:117891 USPATFULL
TITLE: Up-converting reporters for biological and other **assays** using laser excitation techniques
INVENTOR(S): Zarling, David A., Menlo Park, CA, United States
Rossi, Michel J., Lausanne, Switzerland
Peppers, Norman A., Belmont, CA, United States
Kane, James, Lawrenceville, NJ, United States
Faris, Gregory W., Menlo Park, CA, United States
Dyer, Mark J., San Jose, CA, United States
Ng, Steve Y., San Francisco, CA, United States
Schneider, Luke V., Half Moon Bay, CA, United States
PATENT ASSIGNEE(S): SRI International, Menlo Park, CA, United States (U.S. corporation)

	NUMBER	DATE

PATENT INFORMATION:	US 5698397	19971216
APPLICATION INFO.:	US 1995-482203	19950607 (8)
DOCUMENT TYPE:	Utility	
PRIMARY EXAMINER:	Jones, W. Gary	
ASSISTANT EXAMINER:	Fredman, Jeffrey	
LEGAL REPRESENTATIVE:	Morgan, Lewis & Bockius LLP	
NUMBER OF CLAIMS:	11	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	39 Drawing Figure(s); 31 Drawing Page(s)	
LINE COUNT:	3493	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides methods, compositions, and apparatus for performing sensitive detection of analytes, such as biological macromolecules and other analytes, by labeling a probe molecule with an up-converting label. The up-converting label absorbs radiation from an illumination source and emits radiation at one or more higher frequencies, providing enhanced signal-to-noise ratio and the essential elimination of background sample autofluorescence. The methods, compositions, and apparatus are suitable for the sensitive detection of multiple analytes and for various clinical and environmental sampling techniques.

L8 ANSWER 61 OF 92 USPATFULL
ACCESSION NUMBER: 97:115397 USPATFULL
TITLE: Macrocyclic complexes of yttrium, the **lanthanides** and the actinides having peripheral coupling functionalities
INVENTOR(S): Vallarino, Lidia M., 1009 West Ave., Richmond, VA, United States 23220
Leif, Robert C., 5648 Toyon Rd., San Diego, CA, United States 92115

	NUMBER	DATE
PATENT INFORMATION:	US 5696240	19971209
APPLICATION INFO.:	US 1994-351827	19941208 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1991-669833, filed on 15 Mar 1991, now patented, Pat. No. US 5373093, issued on 13 Dec 1994
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Hollinden, Gary E.
ASSISTANT EXAMINER: Hartley, Michael G.
LEGAL REPRESENTATIVE: Hibnick, Gerald R.
NUMBER OF CLAIMS: 16
EXEMPLARY CLAIM: 1
LINE COUNT: 2487
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Symmetrically di-functionalized water soluble macrocyclic complexes of **lanthanide**, actinide and yttrium ions were obtained by metal templated, Schiff-base, cyclic condensation of: (1) a functionalized 1,2-diaminoethane and a dicarbonyl compound selected from the group consisting of 2,6-dicarbonylpyridine, 2,6-diformylpyridine, 2,5-dicarbonylfuran, 2,5-diformylfuran, 2,5-dicarbonyl-thiophene and 2,5-diformylthiophene; or (2) 1,2-diaminoethane and a ring-substituted heterocyclic dicarbonyl compound selected from a group consisting of substituted 2,6-dicarbonylpyridine, substituted 2,6-diformylpyridine, substituted 2,5-dicarbonylfuran, substituted 2,5-diformylfuran; substituted 2,5-dicarbonyl thiophene, and substituted 2,5-diformylthiophene. Asymmetrically functionalized water soluble macrocyclic complexes of the **lanthanide**, actinide and yttrium ions were obtained by metal templated, Schiff-base, cyclic condensation of appropriately substituted diamine and dicarbonyl precursors, with such precursors contributing two heteroaromatic moieties (pyridine, furan, thiophene, or a combination thereof) to the resulting macrocyclic structure. The coordination complexes thus formed are kinetically stable in dilute aqueous solution. They are further reacted, or coupled, through a substituent on the 1,2-diaminoethane or on the pyridine, furan, or thiophene moieties, to one of the following: proteinaceous materials, polysaccharides, polynucleotides, other biologically compatible macromolecules or bridging molecules which, can be further reacted or coupled to the above mentioned substrates. These macrocyclic complexes are suitable in the preparation of reporter molecules and for magnetic resonance, radiation imaging and radiation therapy.

L8 ANSWER 65 OF 92 USPATFULL
ACCESSION NUMBER: 97:70876 USPATFULL
TITLE: Luminescent **lanthanide** chelates and methods of use
INVENTOR(S): Selvin, Paul R., Berkeley, CA, United States

	Hearst, John, Berkeley, CA, United States
PATENT ASSIGNEE(S):	The Regents of the University of California, Oakland, CA, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5656433	19970812
APPLICATION INFO.:	US 1996-762288	19961209 (8)
RELATED APPLN. INFO.:	Division of Ser. No. US 1994-269162, filed on 29 Jun 1994, now patented, Pat. No. US 5622821	
DOCUMENT TYPE:	Utility	
PRIMARY EXAMINER:	Marschel, Ardin H.	
LEGAL REPRESENTATIVE:	Osman, Richard Aron	
NUMBER OF CLAIMS:	20	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	6 Drawing Figure(s); 4 Drawing Page(s)	
LINE COUNT:	1243	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides **lanthanide** chelates capable of intense luminescence. The celates comprise a **lanthanide** chelator covalently joined to a **coumarin**-like or quinolone-like sensitizer. Exemplary sensitizers include 2- or 4-quinolones, 2- or 4-**coumarins**, or derivatives thereof e.g. carbostyril 124 (7-amino-4-methyl-2-quinolone), **coumarin** 120 (7-amino-4-methyl-2-**coumarin**), **coumarin** 124 (7-amino-4-(trifluoromethyl)-2-**coumarin**), aminomethyltrimethylpsoralen, etc.

The chelates form high affinity complexes with **lanthanides**, such as terbium or europium, through chelator groups, such as DTPA. The chelates may be coupled to a wide variety of compounds to create specific labels, probes, diagnostic and/or therapeutic reagents, etc. The chelates find particular use in resonance energy transfer between chelate-**lanthanide** complexes and another luminescent agent, often a fluorescent non-metal based resonance energy acceptor. The methods provide useful information about the structure, conformation, relative location and/or interactions of macromolecules.

L8 ANSWER 81 OF 92 USPATFULL
ACCESSION NUMBER: 90:96767 USPATFULL
TITLE: Fluorescence **immunoassay** using water insoluble dyes
INVENTOR(S): Wagner, Daniel B., Raleigh, NC, United States
Vonk, Glenn P., Fuquay-Varina, NC, United States
Mercolino, Thomas J., Chapel Hill, NC, United States
PATENT ASSIGNEE(S): Becton, Dickinson and Company, Franklin Lakes, NJ, United States (U.S. corporation)

	NUMBER	DATE	
PATENT INFORMATION:	US 4978625	19901218	
APPLICATION INFO.:	US 1987-109689	19871019	(7)

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Kepplinger, Esther L.
ASSISTANT EXAMINER: Saunders, David A.
LEGAL REPRESENTATIVE: Brown, Richard E.
NUMBER OF CLAIMS: 18
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 1 Drawing Figure(s); 1 Drawing Page(s)
LINE COUNT: 660
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB In a method for fluorescence **immunoassay** of an analyte, an antianalyte attached to the surface of a solid support is contacted with the analyte and a tracer which includes a substantially water insoluble fluorescent dye occluded in the nonaqueous portion of a sac. After binding reactions involving the antianalyte, analyte and tracer, the solid support is separated, excitation light is applied and fluorescence from dye in intact sacs bound to the solid support is measured and compared to fluorescence measured when known quantities of analyte are **assayed**. The invention includes a kit of materials useful in performing an **immunoassay** in accordance with the method of the invention.

L8 ANSWER 82 OF 92 USPATFULL
ACCESSION NUMBER: 90:71696 USPATFULL
TITLE: Secondary antibodies against complexes of small molecules and binding partners therefor, their preparation, and their use in diagnostic methods
INVENTOR(S): Self, Colin H., London, United Kingdom
PATENT ASSIGNEE(S): Antibody Technology Limited, United Kingdom (non-U.S. corporation)

	NUMBER	DATE	
PATENT INFORMATION:	US 4956303	19900911	
APPLICATION INFO.:	US 1987-43377	19870428	(7)

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Hill, Jr., Robert J.
LEGAL REPRESENTATIVE: Jacobs & Jacobs
NUMBER OF CLAIMS: 19
EXEMPLARY CLAIM: 1
LINE COUNT: 768
AB A secondary antibody capable of stabilizing the binding of a small molecule to its binding protein is described which secondary antibody is

capable of binding said binding protein in the presence of an in the absence of the small molecule but is not capable of binding said small molecule in the absence of binding protein. Such antibodies may be obtained by forming a complex between a small molecule and its binding protein, using the complex to raise antibodies and selecting the antibodies. The antibodies may be used in competitive **assays** in which it is desired to improve the binding of a small molecule or labelled small molecule to its binding protein.

L8 ANSWER 85 OF 92 USPATFULL
ACCESSION NUMBER: 89:78671 USPATFULL
TITLE: Analyte detection by means of energy transfer
INVENTOR(S): Stavrianopoulos, Jannis, New York, NY, United States
Rabbani, Elazar, New York, NY, United States
Abrams, Samuel B., New York, NY, United States
Wetmur, James G., Scardsdale, NY, United States
PATENT ASSIGNEE(S): Enzo Biochem, Inc., New York, NY, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 4868103	19890919
APPLICATION INFO.:	US 1986-831250	19860219 (6)

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Brown, Johnnie R.
ASSISTANT EXAMINER: Jay, Jeremy M.
LEGAL REPRESENTATIVE: Mosoff, Serle I.; Tzagoloff, Helen
NUMBER OF CLAIMS: 34
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 7 Drawing Figure(s); 2 Drawing Page(s)
LINE COUNT: 1656
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A method is disclosed to detect the presence of an analyte. The method involves forming a complex comprising the analyte and a binding entity. The binding entity comprises a first partner of an energy transfer system. The complex is then contacted with a reporting entity to form a unit. The reporting entity comprises a second partner of the energy transfer system. The first partner and the second partner are within Furster's radius of each other in the formed unit. The unit is irradiated with energy which can only be absorbed by one of said partners, namely, the energy donor, which then emits fluorescent energy.
Some of this energy is absorbed by the other of said partners, namely, the energy acceptor, which also emits fluorescent energy. However, the fluorescent energy of the energy acceptor is of longer wavelength and in
addition may be of substantially greater duration than the fluorescent energy of the energy donor. The detection of fluorescence at the longer wavelength or after a given time interval verifies the presence of the analyte.

L8 ANSWER 91 OF 92 USPATFULL
ACCESSION NUMBER: 87:4868 USPATFULL
TITLE: Fluorescent labels for **immunoassay**
INVENTOR(S): Hinshaw, Jerald C., Ogden, UT, United States
Toner, John L., Webster, NY, United States
Reynolds, George A., Rochester, NY, United States
PATENT ASSIGNEE(S): Eastman Kodak Company, Rochester, NY, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 4637988	19870120
APPLICATION INFO.:	US 1986-825693	19860203 (6)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1981-279398, filed on 1 Jul

1981, now abandoned
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Schwartz, Richard A.
LEGAL REPRESENTATIVE: Rosenstein, Arthur H.
NUMBER OF CLAIMS: 30
EXEMPLARY CLAIM: 28
LINE COUNT: 972
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB There are described stable fluorescent **labels** comprising a complex of **lanthanide** metal and a chelating agent comprising a nucleus which is a triplet sensitizer having a triplet energy greater than that of said **lanthanide** metal and at least two heteroatom-containing groups which form coordinate complexes with **lanthanide** metals and a third heteroatom-containing group or heteratom in or appended to the triplet sensitizer. Labeled physiologically active materials useful in specific binding **assays** such as labeled antigens, heptens, antibodies, hormones and the like comprising the stable fluorescent labels having physiologically active materials adsorbed or bonded thereto are also described.

L8 ANSWER 90 OF 92 USPATFULL
ACCESSION NUMBER: 87:40012 USPATFULL
TITLE: Phenolic fluorescent labels
INVENTOR(S): Hinshaw, Jerald C., Ogden, UT, United States
Toner, John L., Webster, NY, United States
Reynolds, George A., Rochester, NY, United States
PATENT ASSIGNEE(S): Eastman Kodak Company, Rochester, NY, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 4670572	19870602
APPLICATION INFO.:	US 1986-825009	19860203 (6)

RELATED APPLN. INFO.: Division of Ser. No. US 1981-279398, filed on 1 Jul 1981, now abandoned
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Schwartz, Richard A.
LEGAL REPRESENTATIVE: Everett, John R.
NUMBER OF CLAIMS: 10
EXEMPLARY CLAIM: 10
LINE COUNT: 755
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB There are described stable fluorescent **labels** comprising a complex of **lanthanide** metal and a chelating agent comprising a nucleus which is a triplet sensitizer having a triplet energy greater than that of said **lanthanide** metal and at least two heteroatom-containing groups which form coordinate complexes with **lanthanide** metals and a third heteroatom-containing group or heteratom in or appended to the triplet sensitizer. Labeled physiologically active materials useful in specific binding **assays** such as labeled antigens, heptens, antibodies, hormones and the like comprising the stable fluorescent labels having physiologically active materials adsorbed or bonded thereto are also described.

L8 ANSWER 89 OF 92 USPATFULL
ACCESSION NUMBER: 87:50469 USPATFULL
TITLE: Homogeneous fluorescence **immunoassay** using a light absorbing material
INVENTOR(S): Wagner, Daniel B., Raleigh, NC, United States
Baffi, Robert A., Raleigh, NC, United States
PATENT ASSIGNEE(S): Becton, Dickinson and Company, Franklin Lakes, NJ, United States (U.S. corporation)

	NUMBER	DATE

	NUMBER	DATE	
PATENT INFORMATION:	US 4680275	19870714	
APPLICATION INFO.:	US 1985-700578	19850211	(6)

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Rosen, Sam
ASSISTANT EXAMINER: Benson, Robert
LEGAL REPRESENTATIVE: Brown, Richard E.
NUMBER OF CLAIMS: 28
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 3 Drawing Figure(s); 3 Drawing Page(s)
LINE COUNT: 689
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method for separation-free solid phase **immunoassay** of an analyte includes contacting an anti-analyte attached to the surface of a solid support with the analyte, a light absorbing material and a fluorescent tracer for the analyte. The resulting mixture is incubated. The method includes applying excitation light to the mixture and time resolved measurement of fluorescence emission from the tracer. All excitation light and fluorescence emission are absorbed by the light absorbing material except that absorbed and emitted by the tracer bound to the anti-analyte whereby the only fluorescence emission detected is from the bound tracer. Since free tracer in the fluid phase of the **assay** medium does not emit fluorescence, separation of the bound and free fractions is unnecessary. The invention includes a kit of materials useful in performing an **immunoassay** in accordance with the method of the invention.

L8 ANSWER 92 OF 92 USPATFULL
ACCESSION NUMBER: 83:8130 USPATFULL
TITLE: Fluorescence spectroscopy **assay** means with fluorescent chelate of a **lanthanide**
INVENTOR(S): Soini, Erkki, Turku, Finland
Hemmila, Ilkka, Turku, Finland
PATENT ASSIGNEE(S): Wallac Oy, Turku, Finland (non-U.S. corporation)

	NUMBER	DATE	
PATENT INFORMATION:	US 4374120	19830215	
APPLICATION INFO.:	US 1980-128621	19800307	(6)

	NUMBER	DATE
PRIORITY INFORMATION:	SE 1979-2079	19790308

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Fagelson, Anna P.
LEGAL REPRESENTATIVE: Fisher, Christen & Sabol
NUMBER OF CLAIMS: 18
EXEMPLARY CLAIM: 1
LINE COUNT: 282
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB An improved method of determining the nature of a substance by fluoroscence spectroscopy wherein a fluorescent marker is coupled to the molecules of the substance comprises the use of a marker having a longer period of fluorescence than those of possible sources of noise and by employing an exciting radiation pulse of short duration so that the fluorescence of the marker is detected after the objectionable sources of fluorescence have ceased; the marker including a fluorescent **lanthanide** chelate complex.

L11 ANSWER 1 OF 3 USPATFULL
ACCESSION NUMBER: 90:96767 USPATFULL
TITLE: Fluorescence **immunoassay** using water insoluble dyes
INVENTOR(S): Wagner, Daniel B., Raleigh, NC, United States
Vonk, Glenn P., Fuquay-Varina, NC, United States
Mercolino, Thomas J., Chapel Hill, NC, United States
PATENT ASSIGNEE(S): Becton, Dickinson and Company, Franklin Lakes, NJ, United States (U.S. corporation)

	NUMBER	DATE	
PATENT INFORMATION:	US 4978625	19901218	
APPLICATION INFO.:	US 1987-109689	19871019	(7)

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Kepplinger, Esther L.
ASSISTANT EXAMINER: Saunders, David A.
LEGAL REPRESENTATIVE: Brown, Richard E.
NUMBER OF CLAIMS: 18
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 1 Drawing Figure(s); 1 Drawing Page(s)
LINE COUNT: 660
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB In a method for fluorescence **immunoassay** of an analyte, an antianalyte attached to the surface of a solid support is contacted with the analyte and a tracer which includes a substantially water insoluble fluorescent dye occluded in the nonaqueous portion of a sac. After binding reactions involving the antianalyte, analyte and tracer, the solid support is separated, excitation light is applied and fluorescence from dye in intact sacs bound to the solid support is measured and compared to fluorescence measured when known quantities of analyte are **assayed**. The invention includes a kit of materials useful in performing an **immunoassay** in accordance with the method of the invention.

TI Fluorescence **immunoassay** using water insoluble dyes

AB In a method for fluorescence **immunoassay** of an analyte, an antianalyte attached to the surface of a solid support is contacted with the analyte and a. . . intact sacs bound to the solid support is measured and compared to fluorescence measured when known quantities of analyte are **assayed**. The invention includes a kit of materials useful in performing an **immunoassay** in accordance with the method of the invention.

SUMM This invention relates to an **immunoassay** of an analyte and materials used therein, and more particularly relates to a method and materials for time resolved **immunoassay** in which a label is encapsulated in a vesicle.

SUMM A variety of **assay** systems which are both rapid and sensitive has been developed to determine the concentration of a substance in a fluid. **Immunoassays** depend on the binding of an antigen or hapten to a specific antibody and have been particularly useful because they give high levels of specificity and sensitivity. These **assays** generally employ one of the above reagents in labeled form, the labeled reagent often being referred to as the tracer,. . .

SUMM **Radioimmunoassay** (RIA) procedures use radioisotopes as labels, provide high levels of sensitivity and reproducibility, and are amenable

to automation for rapid. . .

SUMM Enzymes have also been used as labels in **immunoassay**. Enzyme **immunoassay** (EIA) wherein an enzyme label is encapsulated in a vesicle later lysed by complement is disclosed in U.S. Pat. No.. . .

SUMM **Fluoroimmunoassay** (FIA), in contrast to EIA, provides direct detection of the label. FIA procedures in which the dyes are entrapped or. . . et al., the liposome is disrupted after a binding reaction and the dye is excited by chemiluminescence generated in the **assay** medium. Kung et al. is an agglutination **assay** and requires a large liposome for dye-aided visual detection of an agglutinate.

SUMM Known FIA methods using organic fluorochromes, such as **fluorescein** or **rhodamine** derivatives, have not achieved the high sensitivity of RIA or EIA, largely because of light scattering by impurities suspended in the **assay** medium and by background fluorescence emission from other fluorescent materials present in the **assay** medium. Scattering is particularly troublesome with fluorochromes having a short (50 nm or less) Stoke's shift (the difference between the wavelength of the absorption and emission). For example, the Stoke's shift of **fluorescein** isothiocyanate is only 20-30 nm. Background fluorescence is particularly troublesome when the **assay** medium is serum. The sensitivity of an **assay** in serum may be reduced up to one hundred fold compared to an identical **assay** in buffer.

SUMM The development of time-resolved **fluoroimmunoassay** (TR-FIA) has contributed to overcoming these problems In this procedure, a fluorochrome label with relatively long fluorescence emission decay time. . .

SUMM A class of labels meeting the requirements of TR-FIA is the **lanthanide** chelates. **Lanthanide** ions, such as ions of europium and terbium, though not fluorescent themselves, form highly fluorescent chelates of long Stoke's shift. . . metal ion emits the energy as fluorescence of exceptionally long decay time (1 ms). A discussion of the use of **lanthanide** chelates in TR-FIA is given in Analytical Biochemistry, 137, 335 (1984) and in Clinical Biochemical Analysis 14, 71 (1984).

SUMM U.S. Pat. No. 4,058,732 to Wieder discloses a method and apparatus for use of **lanthanide** chelates and time resolution in analytical fluorescent spectroscopy.

SUMM U.S. Pat. No. 4,283,382 to Frank et al. discloses an improvement in TR-FIA in which a **lanthanide** chelate **label** is incorporated into a polymeric bead lattice to eliminate water-induced quenching of the fluorescence emission of the label.

SUMM U.S. Pat. No. 4,374,120 to Soini et al. discloses increased stability of **lanthanide** chelates achieved by a 1:1:1 chelate of **lanthanide**, .beta.-diketone, and an aminopolycarboxylic acid analogue having a functional group useful for binding the chelate to a protein.

SUMM European Pat. Application EP 0,064,484-A2 discloses a TR-FIA procedure in which the substance to be determined is coupled to a **lanthanide** by an **aminocarboxylic** acid analogue, and, after incubation, the **lanthanide** is split from the substance to be determined and chelated to a .beta.-diketone before detection.

SUMM . . . of Immunological Methods, 100 59 (1987)) describes enhancement of the fluorescent signal in cell labeling experiments by encapsulating water soluble **fluorescein** and **rhodamine** dyes in the aqueous compartment of liposomes, and suggests that intercalating hydrophobic dyes in the liposomal membrane may enable such. . .

SUMM Although **lanthanide** chelate **labels** are useful in FIA, several problems exist. Covalent attachment of the chelating agent to a protein component of the **assay** is a time consuming operation requiring complex chemical reactions under carefully controlled conditions. Such covalent bonding of the chelating agent. .

. in dissociation of the ion. Dissociation is a particular problem when
the labeled protein in the aqueous phase of the **assay** is in very low concentration. It is toward the solution of these problems that
this invention is directed.

SUMM One aspect of the present invention comprises a method for fluorescence **immunoassay** of an analyte in a fluid. An antianalyte affixed to a solid support is contacted with the fluid and a. . . Fluorescence is measured and compared with the magnitude of emission measured when one or more know quantities of analyte is **assayed** under essentially identical conditions.

SUMM The preferred dye is a fluorescent chelated **lanthanide** ion which is substantially water insoluble and which is occluded in the nonaqueous portion of a sac, most preferably in the lipid portion of a liposome. The most preferred dye is a **lanthanide** ion chelated with a .beta.-diketone, wherein measurement of fluorescence is performed
by time resolution.

SUMM . . . embodiment of the method, substantially all of the analyte binds to both the antianalyte and the tracer in a sandwich **assay** . In another preferred embodiment of the method, the tracer and the analyte compete for an insufficient number of antianalyte binding sites in a competitive **assay**.

SUMM . . . are conjugated by standard methods to a ligand, such as an antigen or antibody, they may be used in an **immunoassay** for an analyte. In contrast to conventional liposome-based **assays**, the method of the invention does not include rupture of the sac. Instead, measurement of fluorescence is carried out subsequent. . .

SUMM . . . the method of the invention are elimination of any chemical manipulation of the dye so that dyes, as for example, **Rhodamine** 800 (Raue et al., Heterocycles 21, 167 (1984)) may be used in spite of their water insolubility and lack of a functional group suitable for conjugation. Further, fluorescent, water-insoluble **lanthanide** chelates, such as the .beta.-diketone chelates, can be taken up into liposomes and used in an **assay** directly in contrast to prior art methods in which water soluble **lanthanide** chelates of low fluorescence are taken up into the aqueous phase of a liposome, followed
by liposome lysis and a. . . the present invention may also be compared with prior art methods in which europium ions are conjugated directly to an **assay** ligand by complex chemical synthesis giving tracers having low ratios of europium ion to ligand.

DRWD The Figure is a plot of concentration versus fluorescence for **assay** of human chorionic gonadotropin in accordance with the method of the invention.

DETD . . . solid support. As known in the art, the solid support may be any support which does not interfere with the **assay**. Exemplary of solid supports which may be used are glass and polymeric materials, such as polyethylene, polystyrene and the like.. . .

DETD The quantity of antianalyte to be attached to the solid support depends on the type of **assay** to be carried out. In a competitive **immunoassay**, a limited amount of antianalyte is attached, whereby insufficient binding sites are available and the analyte and a tracer for the analyte, described below, compete for the available sites. In a sandwich **assay**, excess antianalyte is attached whereby essentially all analyte is bound to the antianalyte.

DETD In performing an **assay** in accordance with the method of the invention, the antianalyte attached to the solid support is contacted with a fluid containing an unknown quantity of analyte, and the **assay** medium is incubated as described below to induce an immunological reaction between the analyte and antianalyte. A tracer for
the. . .

DETD . . . are occluded in the lipid portion of the sac during formation of the sac. Exemplary of such dyes are the **carbocyanines**,

perylenes, and styryls such as 4-dicycano-methylene-2-methyl-6-(p-dimethylaminostyryl)4H-pyran. Particularly preferred dyes are fluorescent chelates of **lanthanide** ions, for example ions of terbium, samarium, and, most preferably, europium.

DETD **Lanthanide** ions are only weakly fluorescent until chelated with an appropriate organic molecule. Any organic molecule may be used which provides. . . be used are benzoylacetone, dibenzoylmethane, thenoyltrifluoroacetone, benzoyltrifluoroacetone, naphthoyltrifluoroacetone, acetylacetone, trifluoroacetylacetone, hexafluoroacetylacetone, and the like. Chelation of the .beta.-diketone with the **lanthanide** ion is routinely carried out by incubating the reagents for an appropriate time. The quantity of **lanthanide** chelate to be used in preparation of tracer depends on the type of **assay** to be carried out and the quantity of analyte in the fluid, and is well known to those of ordinary. . .

DETD . . . analogue thereof constitutes the tracer. The ligand portion of the tracer may be chosen in accordance with the type of **assay** to be carried out. In a competitive **assay**, the ligand to be conjugated to the label is preferably the analyte. In a sandwich **assay**, the ligand portion of the tracer may be any ligand specific for the analyte, and as such may be a. . .

DETD As indicated above, the **assay** medium containing the supported antianalyte, the fluid containing the analyte, and the tracer may be incubated at any temperature and. . .

DETD . . . by exciting the bound label with electromagnetic radiation of suitable wavelength and measuring fluorescence emission. When the dye is a **lanthanide** chelate, excitation radiation is preferably applied as a pulse and emission is preferably measured by time resolution.

DETD In a competitive **assay**, the magnitude of the fluorescence is directly proportional to the quantity of bound tracer and therefore is inversely proportional to the quantity of analyte present in the fluid. In a sandwich **assay**, the concentration of the analyte present in the fluid is directly proportional to the magnitude of light emission. The concentration of the analyte in the fluid may be determined by comparing the magnitude of fluorescence measured upon **assay** of the analyte with the fluorescence measured upon **assay** of a range of known quantities of the analyte **assayed** under essentially identical conditions.

DETD . . . accordance with another aspect of the invention, there is provided a reagent kit or package of materials for accomplishing an **assay** for an analyte in accordance with the method of the invention. The kit may include a solid support having attached. . . such as buffers, saline or other labeled or unlabeled specific antigens, antibodies or complexes thereof useful in carrying out the **assay**. The components of the kit may be supplied in separate containers, as, for example, vials, or two or more of. . .

DETD **Assay** for Human Chorionic Gonadotropin (hCG) Using Fluorescent Europium Liposomes

DETD . . . uL/well) was added and the strips allowed to stand at 4.degree. C. overnight. The blocked strips were washed with HEPES **assay** buffer (HEPES 50 mM, sodium chloride 9 g/L, BSA, 0.5%, sodium azide 0.2 g/L, pH 7.7; 200 uL/well), and stored. . .

DETD 3. **Assay** Procedure

DETD Serum standards of hCG (LKB, Gaithersburg, MD) were aliquoted into microtiter strips (20 uL/well) and diluted with HEPES **assay** buffer (200 uL/well). The strips were shaken for 1.5 h, and washed with HEPES **assay** buffer (6X). A solution of liposomes (250 uL) in HEPES **assay** buffer (10 mL) was added (200 uL/well) and the strips shaken 1.25 h. The strips were washed with HEPES **assay** buffer (6X) and read in an Arcus (LKB) fluorometer. A standard curve plotting concentration versus fluorescence was constructed in which. . .

DETD Thus, in accordance with the invention, a method for fluorescence **immunoassay** includes use of a tracer having a fluorescent dye occluded in the nonaqueous portion of a sac. Because the dyes. . . flexibility opens up the way to the use of dyes which have their maximum emission in the near IR in **assays** of reduced background using inexpensive lasers in cell sorters and use of modern, inexpensive, solid-state light detectors.

CLM What is claimed:

. . . and with a tracer comprising a sac with aqueous and nonaqueous portions, said sac including a substantially water insoluble fluorescent **lanthanide** chelate occluded substantially in the nonaqueous portion of said sac whereby said analyte binds to said antianalyte and said tracer. . . (b) separating said support having bound fraction thereon from said liquid, said bound fraction including an intact sac with said **lanthanide** chelate therein; (c) exciting **lanthanide** chelate in said intact sac by applying electromagnetic radiation thereto; (d) detecting fluorescence from said **lanthanide** chelate; and (e) determining the quantity of said analyte in said liquid by comparing the magnitude of said fluorescence with. . .

7. The method in accordance with claim 1 wherein said **lanthanide** chelate includes an ion selected from the group consisting of europium, terbium, and samarium, said ion being chelated to a. . .

9. The method in accordance with claim 1 further comprising a water soluble **lanthanide** chelate encapsulated substantially in the aqueous compartment of said sac.

. . . an unknown quantity of an analyte and a tracer, said tracer comprising a liposome occluding a substantially water insoluble fluorescent **lanthanide** chelate substantially in the lipid portion thereof, said liposome being conjugated to one of said analyte and a ligand specific. . .

13. A kit of materials for performing an **assay** for an unknown quantity of an analyte in a liquid comprising (1) a solid support having attached thereto an antianalyte specific to an analyte and (2) a tracer for said analyte, said tracer comprising a substantially water insoluble fluorescent **lanthanide** chelate occluded in a nonaqueous portion of a sac having aqueous and nonaqueous portions, said tracer being conjugated to one. . .

18. The kit in accordance with claim 13 further comprising a water soluble **lanthanide** chelate encapsulated substantially in an aqueous compartment of said medium.

L11 ANSWER 2 OF 3 USPATFULL

ACCESSION NUMBER:	87:50469 USPATFULL
TITLE:	Homogeneous fluorescence **immunoassay** using a light absorbing material
INVENTOR(S):	Wagner, Daniel B., Raleigh, NC, United States Baffi, Robert A., Raleigh, NC, United States
PATENT ASSIGNEE(S):	Becton, Dickinson and Company, Franklin Lakes, NJ, United States (U.S. corporation)

	NUMBER	DATE	
PATENT INFORMATION:	US 4680275	19870714	
APPLICATION INFO.:	US 1985-700578	19850211	(6)
DOCUMENT TYPE:	Utility		
PRIMARY EXAMINER:	Rosen, Sam		
ASSISTANT EXAMINER:	Benson, Robert		
LEGAL REPRESENTATIVE:	Brown, Richard E.		
NUMBER OF CLAIMS:	28		

EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 3 Drawing Figure(s); 3 Drawing Page(s)
LINE COUNT: 689
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method for separation-free solid phase **immunoassay** of an analyte includes contacting an anti-analyte attached to the surface of a solid support with the analyte, a light absorbing material and a fluorescent tracer for the analyte. The resulting mixture is incubated. The method includes applying excitation light to the mixture and time resolved measurement of fluorescence emission from the tracer. All excitation light and fluorescence emission are absorbed by the light absorbing material except that absorbed and emitted by the tracer bound to the anti-analyte whereby the only fluorescence emission detected is from the bound tracer. Since free tracer in the fluid phase of the **assay** medium does not emit fluorescence, separation of the bound and free fractions is unnecessary. The invention includes a kit of materials useful in performing an **immunoassay** in accordance with the method of the invention.

TI Homogeneous fluorescence **immunoassay** using a light absorbing material

AB A method for separation-free solid phase **immunoassay** of an analyte includes contacting an anti-analyte attached to the surface of a solid support with the analyte, a light. . . whereby the only fluorescence emission detected is from the bound tracer. Since free tracer in the fluid phase of the **assay** medium does not emit fluorescence, separation of the bound and free fractions is unnecessary.

The invention includes a kit of materials useful in performing an **immunoassay** in accordance with the method of the invention.

SUMM This invention relates to an **immunoassay** of an analyte and materials used therein, and more particularly relates to a method and materials for **immunoassay** which does not require separation of bound and free fractions.

SUMM A variety of **assay** systems which are both rapid and sensitive has been developed to determine the concentration of a substance in a fluid. **Immunoassays** depend on the binding of an antigen or hapten to a specific antibody and have been particularly useful because they give high levels of specificity and sensitivity. These **assays** generally employ one of the above reagents in labeled form, the labeled reagent often being referred to as the tracer. **Immunoassay** procedures may be carried out in solution or on a solid support and may be either heterogeneous or homogeneous. Heterogeneous **assays** require a separation of bound tracer from free (unbound) tracer. Homogeneous **assays** do not require a separation step and thereby provide significant advantage in speed, convenience and ease of automation over heterogeneous **assays**.

SUMM **Radioimmunoassay** (RIA) procedures use radioisotopes as labels, provide high levels of sensitivity and reproducibility, and are amenable to automation for rapid. . . samples. However, all RIA procedures require a separation step, since the parameter measured (nuclear decay) cannot be controlled by changing **assay** conditions or components. In addition, isotopes are costly, have relatively short shelf lives, require expensive and complex equipment, and extensive. . .

SUMM Enzymes have also been used as labels in **immunoassay**. **Enzymeimmunoassay** (EIA) may be homogeneous and does not require precautions against radioactivity. Conjugation of an enzyme with a protein is usually. . .

SUMM Some of the above disadvantages associated with RIA or EIA have been overcome by use of fluorochromes as labels in **immunoassay**. **Fluoroimmunoassay** (FIA) provides direct detection of the label and is readily adaptable to homogeneous **assay** procedures. However, known homogeneous FIA methods using organic fluorochromes, such

as **fluorescein** or **rhodamine** derivatives, have not achieved the high sensitivity of RIA or EIA, largely because of light scattering by impurities suspended in the **assay** medium and by background fluorescence emission from other fluorescent materials present in the **assay** medium. Scattering is particularly troublesome with fluorochromes having a short (50 nm or less) Stroke's shift (the difference between the wavelength of the absorption and emmission). For example, the Stoke's shift of **fluorescein** isothiocyanate in only 20-30 nm. Background fluorescence is particularly troublesome when the **assay** medium is serum. The sensitivity of an **assay** in serum may be reduced up to one hundred fold compared to an identical **assay** in buffer.

SUMM The development of time-resolved **fluoroimmunoassay** (TR-FIA) has contributed to overcoming these problems. In this procedure, a fluorochrome label with a relatively long fluorescence emission decay. . .

SUMM A class of labels meeting the requirements of TR-FIA is the **lanthanide** chelates. **Lanthanide** ions, in particular ions of europium and terbium form highly fluorescent chelates of long Stoke's shift (up to 250 nm). . . metal ion emits the energy as fluorescence of exceptionally long decay time (1 ms). A discussion of the use of **lanthanide** chelates in TR-FIA is given in Analytical Biochemistry, 137 335 (1984).

SUMM U.S. Pat. No. 4,058,732 to Wieder discloses a method and apparatus for use of **lanthanide** chelates and time resolution in analytical fluorescent spectroscopy.

SUMM U.S. Pat. No. 4,283,382 to Frank et al. discloses an improvement in TR-FIA in which a **lanthanide** chelate **label** is incorporated into a polymeric bead lattice to eliminate water-induced quenching of the fluorescence emission of the label.

SUMM U.S. Pat. No. 4,374,120 to Soini et al. discloses increased stability of **lanthanide** chelates achieved by a 1:1:1 chelate of **lanthanide**, .beta.-diketone, and an aminopolycarboxylic acid analogue having a functional group useful for binding the chelate to a protein.

SUMM European patent application EP 0,064,484-A2 discloses a TR-FIA procedure in which the substance to be determined is coupled to an **lanthanide** by an **aminocarboxylic** acid analogue, and, after incubation, the **lanthanide** is split from the substance to be determined and chelated to a .beta.-diketone before detection.

SUMM One aspect of the present invention comprises a method for solid phase **immunoassay** of an analyte without separation of bound and free fractions. An anti-analyte attached to the surface of a solid support. . . material and a tracer for the analyte having an attached label which absorbs and emits light. After incubation of the **assay** mixture, excitation light is applied and light emission is measured by time resolution. The magnitude of light emission is compared with the magnitude of light emission measured when one or more known quantities of analyte is **assayed** under essentially identical conditions.

SUMM . . . method of the invention, the analyte is in a serum sample, the light-absorbing material is an arylaminonaphthalene sulfonic acid, the **tracer** is a **lanthanide** chelate incorporated into a polymeric particle attached to the analyte, and the tracer and the analyte compete for an insufficient. . .

SUMM . . . method of the invention, substantially all of the analyte binds to both the anti-analyte and the tracer in a sandwich **assay**.

SUMM . . . out which does not require a separation of bound and unbound fractions whereby operational simplicity, speed and convenience of homogeneous **assays** is achieved. The label is detected directly without an additional substrate or incubation period to generate a detectable material. The method of the invention provides a highly sensitive homogeneous **assay** essentially free of interfering

emission from other fluorescent materials whereby an analyte present in very low concentration can be accurately. . .

DRWD FIG. 1 shows a tube and other components of use in a competitive **immunoassay** in accordance with the method of the invention;

DRWD FIG. 2 is a standard curve of an **assay** for digoxin in accordance with the method of the invention; and

DRWD FIG. 3 is a standard curve of an **assay** for human chorionic ganadotropin (HCG) in accordance with the method of the invention.

DETD . . . solid support. As known in the art, the solid support may be any support which does not interfere with the **assay**. Exemplary of solid supports which may be used are glass and polymeric materials, such as polyethylene, polystyrene and the like.. . .

DETD The quantity of anti-analyte to be attached to the solid support depends on the type of **assay** to be carried out. In a competitive **immunoassay** as will first be described herein, a limited amount of anti-analyte is attached, whereby insufficient binding sites are available and the analyte and a tracer for the analyte, described below, compete for the available sites. In a sandwich **assay**, as will subsequently be described, excess anti-analyte is attached whereby essentially all analyte is bound to the anti-analyte.

DETD In a competitive **assay** in accordance with the method of the invention, the anti-analyte attached to the solid support is contacted with an unknown quantity of analyte in a fluid, and the **assay** medium is incubated as described below to induce an immunological reaction between the analyte and anti-analyte. A tracer for the analyte is then added, and a subsequent incubation is carried out so that the **assay** medium contains free analyte, free tracer, bound analyte and bound tracer. Alternatively and preferably, the analyte and tracer are added. . .

DETD The tracer provides a means to follow the course of the immunological reaction, and, in a competitive **assay**, preferably consists of a known quantity of the analyte or appropriate analogue thereof coupled to a label. The label may. . . fluorescence emission, which allows detection of the bound tracer without any substantial interference from other light emitting materials in the **assay** system. The most preferred labels absorb excitation light of wavelength about 280 to 375 nm, emit fluorescence of wavelength about. . .

DETD Long decay time fluorescent labels useful in accordance with the method of the invention are pyrene derivatives and, preferably, the **lanthanide** chelates. The latter class of **labels** consists of a **lanthanide** ion, such as an ion of europium or terbium chelated with an organic ligand, as, for example, a .beta.-diketone. Exemplary. . . be used are benzoylacetone, dibenzoylmethane, thenoyltrifluoroacetone, benzoyltrifluoroacetone, naphthoyltrifluoroacetone, acetylacetone, trifluoroacetylacetone, hexafluoroacetylacetone, and the like. Chelation of the .beta.-diketone with the **lanthanide** ion is routinely carried out by incubating the reagents for an appropriate time. The quantity of **lanthanide** chelate to be used in preparation of the tracer depends on the type of **assay** to be carried out and the quantity of analyte in the fluid, and is well known to those of ordinary. . .

DETD The **lanthanide** chelate **label** may be coupled directly to the analyte by conventional means to produce the tracer. Alternatively and preferably, the label is. . .

DETD The **assay** medium containing the supported anti-analyte, the fluid containing the analyte, and the tracer may be incubated at any temperature and. . .

DETD A light absorbing material is added to the **assay** system either before or after the immunological reaction, and may be any material which has an absorption band which overlaps. . . arylaminonaphthalene sulfonic acids, as, for example, 2-(p-anisidinyl) naphthalene-6-sulfonic acid or 1-anilinononaphthalene-8-sulfonic acid (1,8-ANS). The

concentration of absorbing material in the **assay** medium may be from about 10.sup.-2 to 10.sup.-6 M, preferably from about 10.sup.-3 to 10.sup.-4 M.

DETD . . . light absorbing material. The excitation light preferably has a wavelength within the absorption range of the label used. When the **label** is a **lanthanide** chelate, the excitation light preferably has a wavelength of from about 280 to 375 nm, most preferably about 343 nm. . .

DETD . . . 0.5 ms from completion of a pulse. The wavelength of the light emission depends on the label used. When the **label** is a **lanthanide** chelate, the emission wavelength generally is from about 580 to 630 nm. Most preferably, emission is measured at a wavelength. . .

DETD The light absorbing material in the fluid phase of the **assay** system absorbs all excitation and/or emission light which passes through the fluid phase and thereby effectively prevents detection of emission. . .

DETD The method and components of the invention are depicted in FIG. 1 wherein **assay** system 10 includes a preferably polystyrene tube 13 having an open end 11 and a closed end 12. Tube 13. . .

DETD . . . the light source is off. Short decay time background light emission associated with serum or other fluorescent materials in the **assay** medium, including that associated with the solid support itself, is essentially eliminated by time-resolved measurement of emission. Thus, in accordance. . .

DETD In a competitive **assay** as hereinabove described, the magnitude of the light emission is directly proportional to the quantity of bound tracer and therefore. . . The concentration of the analyte in the fluid may be determined by comparing the magnitude of light emission measured upon **assay** of the analyte with the emission measured upon **assay** of a range of known quantities of the analyte **assayed** under essentially identical conditions.

DETD The method of the invention may be adapted to a solid phase sandwich **assay**. This type of **assay** is particularly useful for **assay** of a macromolecular analyte, as, for example, a protein. Any modification of solid phase sandwich **assay** may be used. For example, the anti-analyte may be attached to the solid support in sufficient quantity to bind essentially. . .

DETD . . . absorbing material and the preferred method for excitation and detection of emission may be as described above for the competitive **assay**. However, in the sandwich **assay** of this embodiment of the invention, the concentration of the analyte present in the fluid is directly proportional to the. . .

DETD . . . accordance with another aspect of the invention, there is provided a reagent kit or package of materials for accomplishing an **assay** for an analyte in accordance with the method of the invention. The kit may include a solid support having attached. . . include other reagents, such as other labeled or unlabeled specific antigens, antibodies or complexes thereof useful in carrying out the **assay**. The components of the kit may be supplied in separate containers, as, for example, vials, or two or more of. . .

DETD The following examples of a model system and an **assay** for digoxin are provided to further describe the invention, but are not intended in any way to be limitative of. . .

DETD Thus, in accordance with the invention, a method for solid phase **fluoroimmunoassay** includes addition of a light absorbing material to the fluid phase of the **assay** system. The light absorbing material absorbs all excitation and/or emitted light except that absorbed by and emitted from bound tracer,. . . free tracer. Separation of the bound and free fractions is thus avoided and the simplicity and convenience of a homogeneous **assay** is gained. By measuring light emission from the bound tracer using time resolution,

interference from reflected excitation light and from light scattering and background emission can be minimized whereby higher **assay** sensitivity can be achieved. The method is easily adaptable to all modifications of solid phase competitive and sandwich type **assay** systems. The invention includes a kit of **assay** materials which can be used for either manual or automated **assay**.

CLM What is claimed is:

7. The method in accordance with claim 1 wherein said fluorescent dye is a **lanthanide** chelate.

8. The method in accordance with claim 7 wherein said **lanthanide** chelate is incorporated into a polymeric particle.

. . . an arylaminonaphthalene sulfonic acid, and a tracer consisting of said analyte having attached thereto a particle having incorporated therein a **lanthanide** chelate **label**; (b) incubating said mixture; (c) applying to said mixture through said solid support a pulse of excitation light of wavelength. . .

22. The method in accordance with claim 20 wherein said **lanthanide** chelate is a europium or terbium ion chelated to a .beta.-diketone.

. . . tracer consisting of a ligand specific for said analyte, said ligand having attached thereto a particle having incorporated therein a **lanthanide** chelate **label**; (b) incubating said mixture; (c) applying to said mixture, through said solid support, a pulse of excitation light of wavelength. . .

26. The method in accordance with claim 23 wherein said **lanthanide** chelate is a europium or terbium ion chelated to a .beta.-diketone.

27. A kit of materials for performing an **assay** for an unknown quantity of an analyte in a fluid comprising a light transmitting solid support having attached thereto an. . .

L11 ANSWER 3 OF 3 USPATFULL

ACCESSION NUMBER:	83:8130 USPATFULL	
TITLE:	Fluorescence spectroscopy **assay** means with fluorescent chelate of a **lanthanide**	
INVENTOR(S):	Soini, Erkki, Turku, Finland Hemmila, Ilkka, Turku, Finland	
PATENT ASSIGNEE(S):	Wallac Oy, Turku, Finland (non-U.S. corporation)	
	NUMBER	DATE
PATENT INFORMATION:	US 4374120	19830215
APPLICATION INFO.:	US 1980-128621	19800307 (6)
	NUMBER	DATE
PRIORITY INFORMATION:	SE 1979-2079	19790308
DOCUMENT TYPE:	Utility	
PRIMARY EXAMINER:	Fagelson, Anna P.	
LEGAL REPRESENTATIVE:	Fisher, Christen & Sabol	
NUMBER OF CLAIMS:	18	
EXEMPLARY CLAIM:	1	
LINE COUNT:	282	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB An improved method of determining the nature of a substance by fluoroscence spectroscopy wherein a fluorescent marker is coupled to the molecules of the substance comprises the use of a marker having a longer period of fluorescence than those of possible sources of noise and by employing an exciting radiation pulse of short duration so that the

fluorescence of the marker is detected after the objectionable sources of fluorescence have ceased; the marker including a fluorescent **lanthanide** chelate complex.

TI Fluorescence spectroscopy **assay** means with fluorescent chelate of a **lanthanide**

AB . . . the fluorescence of the marker is detected after the objectionable sources of fluorescence have ceased; the marker including a fluorescent **lanthanide** chelate complex.

SUMM The organic markers used in biological systems, for instance **fluoresceinisothiocyanate** (FITC), **rhodamines**(RBITC, TRITC, RB-200-SC), dansil chloride (DNS-C1), fluorescamine (FL) etc. give a rather strong fluorescence. They are bound covalently to the antibody/antigen. . .

SUMM The fluorescence properties of certain **lanthanide** chelates, especially chelates of europium and terbium, are well suited fluorescent markers. The absorbance of these chelates is very strong,. . .

SUMM . . . to .beta.-diketones the lasering properties of different salicylate chelates have previously been investigated and different methods for fluorometric determination of **lanthanide** ions (Eu, Tb, Sm, Dy) has been developed using these compounds and other ligands, such as terbium with dipicolinic acid. . .

SUMM The strong fluorescence of the **lanthanide** chelates is due to the absorption by the ligands of the excitation radiation and of the energy transfer from the. . .

DETD . . . although with a decreased stability. The aminopolycarboxylic acids used as a secondary ligand in many fluorometric determination systems to determine **trace** amounts of **lanthanides** in water solutions.

DETD In fluoro **immunoassay** systems the europium or terbium ion could be bound to the antibody/antigen or a haptenic molecule via an aminopolycarboxylic acid. . .

CLM What is claimed is:

4. Method according to claim 1, wherein the **aminocarboxylic** acid analogue is an EDTA-analogue.

5. Method according to claim 1, wherein the **aminocarboxylic** acid analogue is a HEDTA-analogue.

17. In a method for the **fluoroimmunoassay** of a substance by means of fluorescence spectroscopy in which a fluorescent marker is coupled to the molecules of the. . .

(FILE 'HOME' ENTERED AT 16:19:38 ON 04 JAN 2001)

FILE 'EMBASE, SCISEARCH, BIOSIS, MEDLINE, USPATFULL' ENTERED AT 16:20:05 ON 04 JAN 2001

L1 1573 S ?ASSAY? AND (LANTHANIDE OR NEODYNIUM OR YTTERBIUM OR ERBIUM)
L2 1693 S ?ASSAY? AND (LANTHANIDE? OR NEODYNIUM OR YTTERBIUM OR ERBIUM)
L3 993 S (LANTHANIDE? OR NEODYNIUM OR YTTERBIUM OR ERBIUM) (5A) (LABEL
L4 277 S L2 AND L3
L5 923 S (LANTHANIDE? OR NEODYNIUM OR YTTERBIUM OR ERBIUM) (5P) ((SENS
L6 107 S L5 AND L3
L7 95 S L6 AND L2
L8 92 DUP REM L7 (3 DUPLICATES REMOVED)
L9 0 S L8 AND ((POLYAMINOCARBOXYLIC) OR (PYRIDINE CARBOXYLIC) (W) A
L10 3 S L8 AND ((AMINOCARBOXYLIC) OR (PYRIDINE CARBOXYLIC) (W) ACID?
L11 3 DUP REM L10 (0 DUPLICATES REMOVED)